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A STUDY OF GENETIC VARIABILITY IN CENTRAL INDIA DURUM WHEATS*

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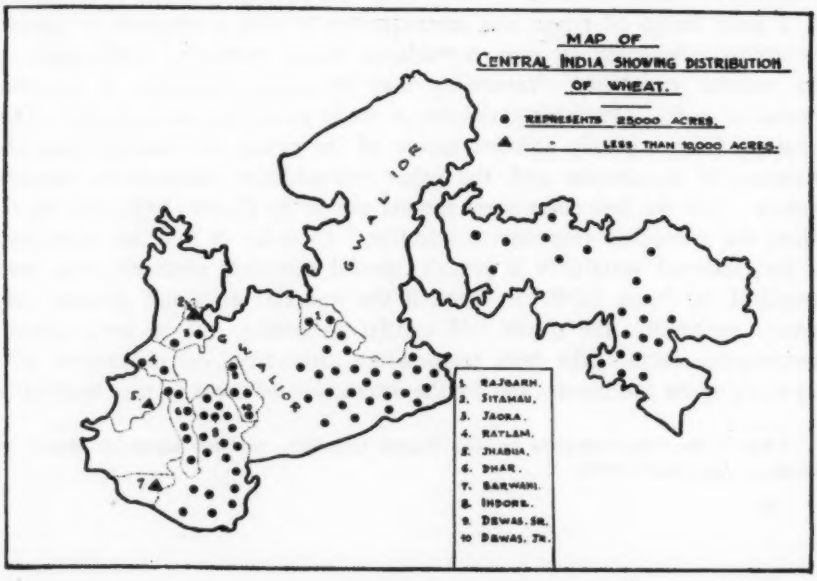
I. Introduction

The indigenous crop is an unselected bulk grown year after year by the cultivators. The action of the Natural Selection would be either to maintain one particular type to the complete exclusion of all others or to maintain a mixture of types in equilibrium. Hutchinson and others (1937, 1938) as a result of studies on cotton and *jowar* are of the opinion that Natural Selection results in the establishment of a well adapted population consisting of a great range of types, and consequently storing a measure of genetic variability. Selection in such population would mean the exploitation of the natural variability. Variability may be either heritable or environmental or both. Heritable variability is made up of two components. One resulting from a purely additive action of the genes, and second from the presence of dominance and the other non-additive interactions between factors. It is the first component termed *genetic* by Fisher (1930) that determines the immediate response to selection. In order to find out what part of the observed variability is genetic, special statistical methods have been described by Panse (1940 *a*). Even if the material does not possess any genetic variability, the plants will exhibit variability due to environment. Innumerable factors like rain, temperature, humidity, soil characters, etc., are working on the plants, and by the interaction of these factors variability

* Part of the thesis submitted to the Nagpur University for the degree of Master of Science in Agriculture (1944).

is created which is entirely environmental. If from a genetically pure population, apparently differing plants are selected and their progenies grown, the mean values of these progenies will approach the mean of the parental population, as the plants from which the progenies are derived exhibited only non-heritable variability. The success of evolving a new strain will depend upon the amount of genetic variability initially present in the material. A comparative study of the genetic variability present in samples obtained from different areas in all important characters related to yield would thus be the best procedure to start with, before embarking on any programme of selection. If there is no genetic variability, there will be no response to selection.

Wheat is the principal *rabi* crop in Central India which is divided into three agriculturally distinct regions:—(1) the Malwa plateau, (2) Nimar or the area south of the Vindhyas and Nimawar tracts and (3) Bundelkhand and Baghelkhand—a tract hilly in parts lying between the black cotton soil of C.P. and the Gangetic alluvium of U.P. The two main kinds of wheat grown in Central India are those belonging to the *vulgare* and *durum* sub-species. A third type *turgidum* is also found to a very small extent on the Malwa plateau. The *vulgare* or *pissi* wheats are grown interspersed with *durum* or *Malvi* wheats and no line of demarcation can be drawn between the tracts of these two wheats. But in certain tracts one type predominates the other. In Malwa plateau and places west of Malwa plateau *durum* wheat predominates, and portions lying south of the U.P. and east of Malwa plateau are grown more with *vulgare* wheats. The total area under wheat in C.I. is roughly about 20 lakh acres as shown in the attached map of C.I.



II. Theory of Estimation of Genetic Variability

Panse (1940 *a*) has shown with regard to the staple length studies in crosses among *G. arboreum* cotton types, that the genetic portion of the variance in a population can be estimated by growing progenies from a number of individuals selected at random from the population and taking out the regression of the progeny means on the parental values. To determine the progeny means accurately a replicated trial is necessary. This regression coefficient then represents the genetic fraction of the variability in the population from which the progenies were obtained.

The rate at which the population will respond to selection will depend upon the amount of genetic variance available, and when the ratio of genetic component to the total variance is at a maximum, selection will be most effective.

III. Experimental Material

Fifteen one pound samples were obtained from the following States in Malwa and Nimar (C.I.). Four of these samples were of red grain and the remaining white. The names of the States with number of samples are given below. (See also map of C.I. on p. 2.)

	Name of the State	No. of sample	Grain colour
1	Rajgarh ..	3	2 white, 1 red
2	Sitamau ..	1	white
3	Jaora ..	1	white
4	Ratlam ..	2	1 white, 1 red
5	Jhabua ..	2	1 white, 1 red
6	Dhar ..	1	white
7	Dewas Junior ..	1	white
8	Dewas Senior ..	1	white
9	Indore ..	1	white
10	Barwani (Nimar) ..	2	1 white, 1 red
	Total ..	15	

These fifteen samples were grown along with an Institute sample in a randomised block layout with five blocks. The plot size was $7' \times 25'$ and the block size $56' \times 50'$, plots being arranged in two rows in each block. Sowing was done on 29th October 1941, by a desi drill with tynes 14" apart at the rate of 50 lbs. per acre. When the germination was complete, a germination count was taken in four experimental lines, leaving one line on either side of the plot and two feet at each end of the plot as non-experimental margins. From each plot, after the stand was made more uniform by thinning the plants wherever they were in bunches and leaving only singles, twenty plants were selected at random for observations and these were properly labelled for further studies. There were thus 100 plants in each

sample. When the crop was ready for harvest, which was about the first week of March 1942, these selected plants were first harvested individually. Number of tillers and ears, weight of grain and straw per plant, number of grains per ear and weight of 100 grains were recorded on each of these plants for all varieties and were later analysed statistically.

Eighteen out of these hundred plants were selected at random from each of ten samples which showed the greatest gross variability in all characters. It was felt that regression line based on 18 points would be reasonably accurate. This number could be increased to 25 or 30 to get a more accurate regression line, but in the present case, it could not be managed. These progenies were then sown in the next season, that is, on 30th October 1942, in three randomised blocks. Progenies were grown from only ten samples including the local sample as the amount of material would have become unmanageable if all sixteen samples were included. Instead of randomising all the 180 (18×10) progenies together in each block, the ten families were first randomised and within each family plot the eighteen progenies were randomised. Each progeny plot consisted of one line of ten plants dibbled six inches apart, the distance between lines being 14″. The progenies were laid out in three rows to make the block as compact as possible. The size of the block was $21' \times 17'$; one point at each end of the plot and one line on either side of the blocks were sown with local wheat as non-experimental margins. The crop was ready for harvest by the middle of March 1943, and the actual harvesting was done on 27th March 1943. Mean number of tillers and ears, weight of grain and straw per plant, mean number of grains per ear and weight of 100 grains were calculated and analysed statistically.

IV. Estimation of Gross Variability

The total variability present for the mean number of tillers and ears per plant, number of grains per ear and weight of 100 grains in each of the sixteen samples is estimated by first calculating the sum of squares from the individual plant values within each plot and pooled over all plots. The corresponding mean squares are then obtained by dividing the sum of squares by the respective total degrees of freedom and are given in Table I. Variances for each character are then tested for their heterogeneity among the different samples by a χ^2 (chi square) test (Stevens, 1936). The values of χ^2 for each character with corresponding values of ρ are shown below:—

Character	χ^2 (chi square)	ρ
Number of tillers per plant =	77·39	< ·01
,, ears =	90·21	< ·01
,, grains per ear =	299·14	< ·01
Weight of 100 grains =	62·55	< ·01

$n = 15$, $\chi^2 = 30{\cdot}578$ for ·01 level

χ^2 (chi square) is highly significant for all characters indicating that the differences in the gross variability among the different samples are highly significant for all characters. For example, the mean square for the number of tillers per plant ranges from 1·67 in Barwani red to 5·25 in Jhabua Kathia; for number of ears per plant from 1·31 in Barwani red to 4·31 in Dhar local; for number of grains per ear from 12·06 in Rajgarh Kotra white to 74·78 in Barwani white and for weight of 100 grains from 0·33 in Barwani white to 1·06 in Rajgarh Kotra white (see also Table I).

TABLE I

Shows total degrees of freedom, mean squares, and arithmetical means of number of tillers and ears per plant, number of grains per ear, and weight of 100 grains of 16 samples

Samples	No. of tillers per plant			No. of ears per plant			No. of grains per ear			Weight of 100 grains (gm.)		
	d. f.	Mean square	Arith. mean	d. f.	Mean square	Arith. mean	d. f.	Mean square	Arith. mean	d. f.	Mean square	Arith. mean
1. Jhabua Kathia ..	92	5·25	4·90	84	3·92	3·76	82	30·13	19·05	82	0·62	4·85
2. Rajgarh Talain white ..	92	5·06	4·16	90	3·67	3·29	88	18·18	17·06	88	0·84	4·98
3. Dhar local ..	86	4·62	5·28	85	4·31	4·15	84	20·47	18·71	84	0·52	4·84
4. Ratlam white ..	89	4·12	3·73	88	2·71	3·17	88	23·99	16·02	88	0·94	4·68
5. Dewas Junior ..	85	4·03	3·91	86	1·80	3·11	85	12·21	15·05	85	0·96	5·00
6. Jhabua Bajia ..	92	3·60	3·76	84	1·65	2·72	80	20·99	15·84	80	0·57	3·90
7. Ratlam red ..	89	3·57	4·24	85	2·87	3·53	84	13·39	14·66	85	0·63	4·82
8. Jaora local ..	90	3·53	3·73	92	2·70	3·08	89	14·85	15·60	89	0·42	4·98
9. Dewas Senior ..	91	3·45	3·92	89	2·86	2·97	89	22·14	16·62	89	0·55	5·16
10. Rajgarh Talain red	91	3·11	3·65	88	2·42	3·24	87	16·11	16·09	87	0·68	5·23
11. Rajgarh Kotra white ..	88	2·82	3·69	86	1·79	3·18	86	12·06	16·37	86	1·06	5·28
12. Indore White ..	91	2·77	3·58	92	2·08	2·86	91	23·67	15·63	91	0·38	4·81
13. Sitamau local ..	90	2·67	3·45	88	2·04	2·98	86	16·47	15·80	86	0·61	4·80
14. Dhar selection ..	89	2·47	3·74	86	1·76	2·75	85	21·95	16·04	85	0·78	5·24
15. Barwani white ..	93	1·72	2·86	90	1·32	2·40	90	74·78	23·12	90	0·33	4·11
16. Barwani red ..	93	1·67	2·81	93	1·31	2·47	90	54·03	22·99	90	0·49	4·19

V. REGRESSION COEFFICIENTS

The gross variability as has been described earlier is partly heritable and partly environmental. In order to find out the genetic fraction of the gross variability for each sample, regression of the progeny means on the parental plant values is calculated by the method described by Panse (1940 *a*). These regression coefficients for each sample and for each character are given in Table II.

Since there are considerable and significant differences in the gross variability among the different samples for all characters, it is expected that

there would be high genetic variability present in the samples. But the regression coefficients show that the variability is mostly environmental and very little of it is of genetic origin as none of the regression coefficients except those of Jhabua Kathia and Barwani white are significant. The other regression coefficients are non-significant and small, and in some cases have negative values also. These negative values are only due to sampling errors and they are not significant in a single case.

TABLE II

Shows coefficients of regression of progeny means on parental values and the standard errors of C.I. durums

Samples	No. of tillers per plant		No. of ears per plant		No. of grains per ear		Weight of 100 grains (gms.)	
	b_1	S. E. of b_1	b_1	S. E. of b_1	b_1	S. E. of b_1	b_1	S E. of b_1
1 Jhabua Kathia ..	+·9951*	·2583	+1·0000†	·3854	+·1463	·1503	+·2636†	·1194
2 Rajgarh Talain white ..	—·0530	·6175	— ·1463	·6798	—·1841	·1342	+·0066	·0565
3 Dhar local ..	+·0112	·3966	+ ·1532	·4223	+·0597	·1823	+·1184	·2428
4 Ratlam white ..	—·0068	·3610	+ ·0771	·3842	+·0113	·2540	+·0503	·0536
5 Indore white ..	—·0908	·3004	+ ·0454	·6114	—·1737	·1587	+·1643	·1400
6 Barwani white ..	+·7453	·3879	+ ·1118	·4374	—·0274	·1109	+·3364†	·1335
7 Ratlam red ..	+·1251	·3343	+ ·3200	·4382	—·2833	·2109	+·0569	·1056
8 Jaora local ..	+·3806	·3284	+ ·5969	·5435	—·0738	·1611	+·0808	·1056
9 Dewas Senior ..	+·0577	·3239	+ ·0508	·4357	+·3248	·1855	—·0189	·0815
10 Rajgarh Kotra white ..	+·1708	·1569	+ ·0836	·2764	—·1262	·1963	+·0292	·0524

* Significant on 1% $n = 15$ $t = 2{\cdot}947$ for ·01

† Significant on 5% $t = 2{\cdot}131$ for ·05

(i) *Number of tillers per plant*

The regression coefficient b_1 is highly significant for Jhabua Kathia and just short of significance in case of Barwani white (Table II). The portion of the total variance which is genetic in these two samples is given in Table III. Ninety-nine per cent. of the total variance is estimated to be genetic in Jhabua Kathia and seventy-five per cent. in Barwani white. These estimates are subject to sampling errors arising from the sampling errors of the estimates of gross variance as well as those of the regression coefficients.

(ii) *Number of ears per plant*

The regression coefficient b_1 is significant on 5% level in case of Jhabua Kathia only, and non-significant and positive in the remaining samples, except in Rajgarh Talain white. The whole of the variance of Jhabua Kathia is apparently genetic, but since the regression coefficient which forms the basis of the calculation of the genetic variance is subject to an appreciable

sampling error, the proper conclusion to be drawn is that a large portion of the total variance in Jhabua Kathia is genetic whereas in Barwani white this portion is only 11%.

(iii) *Number of grains per ear*

In case of samples from Dewas Senior, the regression coefficient b_1 is just short of significance. The genetic variance is about 32% of the total variance. In the remaining samples the coefficient is non-significant and small.

(iv) *Weight of* 100 *grains*

The regression coefficient b_1 is significant on 5% level in Jhabua Kathia and Barwani white samples. In others, it is non-significant, small and either positive or negative. The genetic fraction of the total variance for both samples is given in Table III and is 26% and 34% in Jhabua Kathia and Barwani white respectively.

Table III shows the regression coefficients and the genetic variance.

TABLE III

Sample	Tillers per plant		Ears per plant		No. of grain per ear		Weight of 100 grains (gms.)	
	b_1	Gen. var.	b_1	Gen. var.	b_1	Gen. var	b_1	Gen. var.
Jhabua Kathia..	+·99	5·22	+1·00	3·92	..	..	+·26	·16
Barwani white ..	+·75	1·24	+ ·11	·15	..	..	+·34	·11
Dewas Senior ..	..	..	..	..	+·32	7·08	..	..

Developmental studies have shown that high tiller and ear numbers are associated with high yield. Selection for high tiller and ear numbers can be most effective in Jhabua Kathia which has shown the highest genetic variability in these two characters, and to a lesser extent in Barwani white which shows high genetic variability for tiller number. The Central India *durums* are characterised by a high grain weight and selection for higher grain weight can also be made in these two Central India *durum* samples which contain about 33% genetic variability. These two samples from Barwani and Jhabua States thus seem to offer the best scope for selection for greater yield among the samples tested.

In the remaining attributes, that is, number of grains per ear, these two samples do not show any genetic variability but a third sample from Dewas

Senior shows about 32% genetic variability in this character, and improvement can be possible in this sample for this character, but this sample does not show any variability in other attributes of yield, namely, tiller number, ear number and seed weight, hence the sample does not appear suitable for making further selection for improving the yield.

VI. Discussion

The different samples of local *durum* wheat collected from the States in Central India do not show sufficient variability in all the attributes of yield to make selection effective except in two samples from Jhabua and Barwani States. Improvement by selection, therefore, appears to be possible only in these samples. The small genetic variability in all samples is probably due to the fact that as wheat is the principal *rabi* crop of Central India, the cultivator has been very particular in maintaining the purity of his seed stock by using for seed purposes the same material year after year. In some cases the cultivator also selects well-developed ears individually for his sowing purposes. This repeated selection combined with the fact that wheat is essentially a self-fertilised crop has resulted in the elimination of all variability. The situation is different in the case of another important C.I. crop, namely, cotton which does show a considerable amount of genetic variability among the samples collected in the tract. In this case the cultivator has no control over the seed he uses, as his produce is always sold as seed cotton and he has to buy his seed requirements from the ginneries which usually mix cotton of different tracts before ginning. Moreover, there is a certain amount of cross-pollination in cotton which also results in creating genetic variability.

The large and significant differences in total variability observed in different samples are found to be mainly environmental and of a non-genetic nature. Since the samples were grown under the same environment, differences in variability among the different samples from this source are ordinarily not to be expected. An explanation, therefore, has to be sought for the large differences in environmental variability. The following factors have, however, to be considered in this connection.

(i) *Stand.*—Plants in plots of those samples where the stand is poor will get more room to produce more tillers and ears than in plots where the stand is good. Variability in tiller and ear number between the plants on the plots of former samples is likely to be higher than in plots of those where the stand is optimum.

(ii) *Time of maturity.*—Wheat in Central India is grown as an unirrigated crop and the development of the grain will depend upon the moisture avail-

able to the plants. Early plants will have a normal development of the grain, but in late maturing plants the development of the grain will be affected by the amount of moisture present in different zones of the soil after the critical period in maturity, that is, after the period when plants would normally mature. Due to soil heterogeneity, the amount of moisture will vary in different zones and the plants growing on these zones will have varying degrees of development of grains. Differences in variability for size of grain within such samples will be higher than in those samples where the plants mature earlier and, therefore, more uniform.

(iii) *Time of harvesting*.—If early types are harvested along with the late types, some of the plants in the early types will shed their grains, while others will have their full quota of grains. Variability in the number of grains per ear in the earlier types may thus be higher than between plants of the late types. Variability introduced by this factor is not considerable in this experiment as the varieties were harvested in groups, that is, early varieties were harvested first, then the medium maturing and last of all, the late ones. The ideal procedure to avoid discrepancies due to this factor would have been to harvest each plot as it matured.

In conclusion it should be stated that there is very little genetic variability present in the Central India *durum* wheats and the problem of improving the yield of Central India *durum* wheat can only be tackled successfully, if genetic variability could be created in the material by hybridising the local material with outside strains. The mean values for number of tillers, number of ears per plant and weight of 100 seeds in case of Jhabua Kathia are higher than those of Barwani samples and as such the samples from Jhabua would be more suitable for selection than the Barwani samples. The genetic variability present in samples from Jhabua and Barwani States can, however, be exploited by making extensive field selections in these areas.

VII. Summary

Sixteen samples of the local *durum* wheat from States in Central India were grown in a randomised varietal trial with five blocks for studying the genetic variability in the local *durum* wheats. One hundred plants distributed evenly over all the replicates were labelled after the germination was complete. These plants were, when mature, harvested individually and tillers and ears per plant, number of grains per ear, weight of 100 grains and weight of grain and straw per plant recorded. From these observations, gross variability present in different samples was estimated. It is found that there are significant differences among the samples for gross variability. The following ten out of sixteen samples were selected on the basis of their

gross variability and high mean values for estimating that portion of the variability which is genetic:—

Barwani white, Dhar local, Dewas Senior, Jaora local, Jhabua Kathia, Rajgarh Kotra white, Rajgarh Talain white, Ratlam white, Ratlam red and Indore white. Eighteen progenies from each of these ten samples were grown next year on three randomised blocks. All the attributes of yield were recorded on them and the regression of these progeny means on their parental values were calculated, and the results indicate that there is no genetic variability in most samples except in two, from Jhabua and Barwani States. Most of the gross variability is found to be environmental.

Selection can thus be effective only in Jhabua and Barwani local wheats, while for general improvement, hybridisation of local wheats with outside types would appear necessary.

VIII. Acknowledgements

The investigations dealt with in the present paper were carried out under the supervision and guidance of Mr. K. Ramiah, the then Geneticist and Botanist, Cotton Genetics Research Scheme, Indore, to whom I am highly indebted. I have to thank Dr. V. G. Panse, Statistician, Indian Central Cotton Committee, for his advice on layouts and very helpful criticisms of the statistical interpretation of the results.

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IMPROVEMENTS IN COLORIMETRIC DETERMINATIONS

Part II. A Rapid Method for Estimation of Organic Carbon in Soils

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I. Introduction

The rapid titration methods of Schollenberger (1927, 1931), modified by Walkley and Black (1934) and Walkley (1935) and of Khanna and Sen (1946) for estimation of organic carbon in soils are based on oxidation of organic matter by potassium dichromate and potassium ferricyanide in acidic and alkaline medium respectively. In the former reaction the change of chormate (Cr^{--}) to chromic (Cr^{+++}) and in the latter from ferricyanide (Fe^{+++}) to ferrocyanide (Fe^{++}) takes place in the process of oxidation which can be represented stoichrometrically. Both the methods have been compared, the former by Crowther (1935) at the instance of the Organic Carbon Committee and the latter by Khanna and Sen (*loc. cit.*) with the combustion method and have given fairly reliable results with the help of appropriate correction factors.

During the application of the dichromate method on a large number of soils with high and low content of organic carbon, it was observed that the colour of the titration mixture changed from yellow to green. This colour change was dependent upon the concentration of organic carbon and as the latter increased, there was noticed a gradual increase in the intensity of green colour. At higher concentrations, deep green colours were formed. This led to the standardization of colours in the titration mixtures in relation to the corresponding concentration of organic carbon in soils and the standardised colours were represented on charts for easy reference. The procedure followed in developing this rapid method and results obtained are given below.

II. Procedure and Discussion of Results

Six soil samples, air dried and sieved through 100-mesh varying in organic carbon from 100 mgm. to about 800 mgm. % were weighed 5 gm. each into a 150 c.c. conical flask. 10 c.c. $NK_2Cr_2O_7$ were added, followed by a quick addition of 20 c.c. concentrated H_2SO_4 (Sp. Gr. 1·84). The mixture

was stirred for a few minutes and allowed to cool under the tap for about ten minutes. By this time, a clear supernatant liquid of different shades of yellow, green and their mixture was obtained. 2 c.c. of this supernatant liquid was pipetted into a 100 c.c. flask and made upto volume. 10 c.c. of this solution was taken for colorimetric estimation in a photo-electric (Klett Sommerson) colorimeter using blue light filter and the scale readings noted as standard (Table I).

TABLE I

Standardisation of colours by photo-electric colorimeter

Sl. No.	Sample No.	Og. carbon per 100 gm. of soil (Walkley and Black).	Average scale readings in the colorimeter (Dilution 50 times)
1	636	106	450
2	423	257	300
3	534	391	195
4	453	446	134
5	369	563	105
6	438	778	82

For developing a field method, a simpler micro-technique was followed. 1 gm. of air dried soil was weighed in a test-tube and 2 c.c. of normal dichromate and 4 c.c. of concentrated sulphuric acid added in the manner described above. The mixture was gently shaken or stirred with glass rod and allowed to cool for ten minutes. 2 c.c. of the supernatant liquid was taken in a glass vial and the different colours represented on the chart (Fig. 1) against the known concentration of organic carbon estimated by Walkley and Black's method (Table II).

TABLE II

Showing standard colour chart

Sl. No.	Sample No.	Org. carbon in mgm. per 100 gm. of soil (Walkley & Black).	Colour description	Org. carbon mgm. per 100 gm. of soil
1	636	106	Bright yellow	100–200
2	423	257	Yellow	200–300
3	534	391	Greenish yellow	300–400
4	453	446	Yellowish green	400–500
5	369	563	Dull green	500–600
6	438	778	Bright green	Above 600

In view of the encouraging results obtained, a large number of soils varying in organic carbon content were studied (Table III) both by the combustion and the colorimetric methods.

TABLE III

Comparative results of organic carbon estimation by the two methods

Sl. No.	Sample No.	Org. carbon mgm. 100 per gm. soil (Walkley & Black)	Org. carbon mgm. per 100 gm. soil (Colorimetric method)
1	401	247	256
2	403	351	366
3	404	284	255
4	405	174	182
5	406	304	291
6	407	354	303
7	408	428	451
8	409	377	256
9	410	370	337
10	412	234	232
11	413	274	282
12	416	397	355
13	417	404	397
14	418	94	127
15	451	335	336
16	452	287	230
17	426	515	520
18	454	231	250
19	455	427	404
20	456	335	332
21	457	505	506
22	458	312	348
23	459	202	230
24	460	658	661
25	461	257	202
26	462	414	361
27	356	341	304
28	357	115	117
29	358	358	331
30	359	220	272
31	360	400	403
32	361	79	95
33	362	607	669
34	365	193	128
35	366	1260	1246
36	367	39	85
37	641	507	518
38	633	144	142
39	638	137	135
40	642	615	577
41	7 F	287	286
42	640	217	202
43	639	628	656
44	637	244	262

In the above table columns 3 and 4 give for different soils, the value of organic carbon (in mgm. per 100 gm. of soil) estimated by the method due

to Walkley and Black and the colorimetric method. Analysis of variance of these values is given (Table IV) below:

TABLE IV

Showing analysis of variance

Due to	D.F.	S.S.	M.S.
Soils ..	43	3582771·94	83320·28
Methods ..	1	786·01	786·1
Error (soils x methods). ..	43	26047·49	605·75
Total ..	87	3609605·44	

It will be clear from the above analysis that there is no significant difference between the results given by the two methods. The mean values by the two methods over all 44 soils are 344·57 and 338·59 and the difference between them is not larger than its standard error, which is 5·25. There is a very high correlation between the two methods, the correlation coefficient being ·98. In particular, if one considers the variation between the samples by the colorimetric method, 97% of this variation is accounted for by the regression of the colorimetric value on the standard method. It may be concluded that the colorimetric method reproduces very closely the results given by the standard method.

And the colorimetric method possesses distinct advantages over the Walkley and Black's method. In the latter, the end point is often overshot because of the solution flashing suddenly to green with little or no warning. Besides the ferrous ammonium sulphate solution needs elaborate arrangements for preservation and use of fluoride in presence of sulphuric acid permanently scratches the glass ware used in these estimations. The procedure may also be applied to all the various oxidation methods where dichromate is used as oxidising agent.

In the study of the field method (R.C.M.), the same soils as were used in Table III, were taken by two independent workers at two different times and organic carbon content determined by comparing against the colour standards (Fig. 1) mentioned above. It will be obvious (Table V) that barring two cases of divergence in results marked with asterisk due to different workers and except at lower ranges, the correspondence between the Walkley and Black's methods and the field technique was fairly close to provide, for all practical purposes, an extremely easy and simple means for assessing the value of organic carbon in soils.

TABLE V

Comparison of field technique (R.C.M.) and Walkley and Black's method

Sl. No.	Sample No.	Worker A		Worker B		Organic carbon % (Walkley and Black)
		Colour	Org. carbon mgm. %	Colour	Org. carbon mgm. %	
1	356	Greenish yellow	300–400	Greenish yellow	300–400	341
2	357	Bright yellow	100–200	Bright yellow	100–200	115
3	358	Greenish yellow	300–400	Greenish yellow	300–400	358
4	359	Yellow	200–300	Yellow	200–300	220
5	360	Greenish yellow	300–400	Greenish yellow	300–400	400
6	361	Bright yellow	100–200	Bright yellow	100–200	79
7	362	Bright green	Above 600	Bright green	Above 600	607
8	365	Bright yellow	100–200	Bright yellow	100–200	193
9	366	Bright green	Above 600	Bright green	Above 600	1260
10	367	Bright yellow	100–200	Bright yellow	100–200	39
11	369	Dull green	500–600	Dull green	500–600	521
12	401	Yellow	200–300	Yellow	200–300	247
13	403	Greenish yellow	300–400	Greenisn yellow	300–400	351
14	404	Yellow	200–300	Yellow	200–300	284
15	405	Bright yellow	100–200	Bright yellow	100–200	174
16	406	Greenish yellow	300–400	Yellow	200–300*	304
17	407	Greenish yellow	300–400	Greenish yellow	300–400	354
18	408	Greenish yellow	300–400	Greenish yellow	300–400	428
19	409	Greenish yellow	300–400	Greenish yellow	300–400	377
20	410	Greenish yellow	300–400	Greenish yellow	300–400	370
21	412	Yellow	200–300	Yellow	200–300	234
22	413	Yellow	200–300	Yellow	200–300	274
23	416	Greenish yellow	300–400	Greenish yellow	300–400	397
24	417	Greenish yellow	300–400	Greenish yellow	300–400	404
25	418	Bright yellow	100–200	Bright yellow	100–200	94
26	451	Greenish yellow	300–400	Greenish yellow	300–400	335
27	452	Yellow	200–300	Yellow	200–300	287
28	453	Yellowish green	400–500	Yellowish green	400–500	446
29	454	Yellow	200–300	Yellow	200–300	231
30	455	Greenish yellow	300–400	Greenish yellow	300–400	427
31	456	Greenish yellow	300–400	Greenish yellow	300–400	335
32	457	Yellowish green	400–500	Yellow green	400–500	505
33	458	Yellow	200–300	Yellow	200–300	312
34	459	Yellow	200–300	Yellow	200–300	202
35	460	Dull green	500–600	Dull green	500–600	658
36	461	Yellow	200–300	Yellow	200–300	257
37	462	Yellowish green	400–500	Greenish yellow	300–400*	414
38	633	Bright yellow	100–200	Bright yellow	100–200	144
39	637	Yellow	200–300	Yellow	200–300	244
40	638	Bright yellow	100–200	Bright yellow	100–200	137
41	639	Dull green	500–600	Dull green	500–600	628
42	640	Bright yellow	100–200	Bright green	100–200	217
43	642	Dull green	500–600	Dull green	500–600	615
44	7 F	Yellow	200–300	Yellow	200–300	287

III. SUMMARY

1. The paper describes a rapid method for estimation of organic carbon by photo-electric colorimeter which has distinct advantages over Walkley and Black's wet combustion method. In the procedure followed,

the change of colour from chromate yellow to chromic green in oxidation of organic matter by dichromate and sulphuric acid, has been made use of for colour standardization and estimation of organic carbon in soils.

2. A simple method suitable as field technique (R.C.M.) has also been evolved on the same principle and tested on a large number of soils of different organic carbon concentration with a fair degree of correspondence with Walkley and Black's method.

IV. Acknowledgements

The work was carried out as part of the Sugarcane Research Scheme in Bihar financed jointly by the Bihar Government and the Indian Central Sugarcane Committee to whom grateful thanks are due. The assistance afforded by Mr. K. S. Bandopadhyay, Statistician at the station in analysing data pertaining to Table III and by Mr. Sheodayal Sinha, Junior Research Assistant in analytical work, is also acknowledged.

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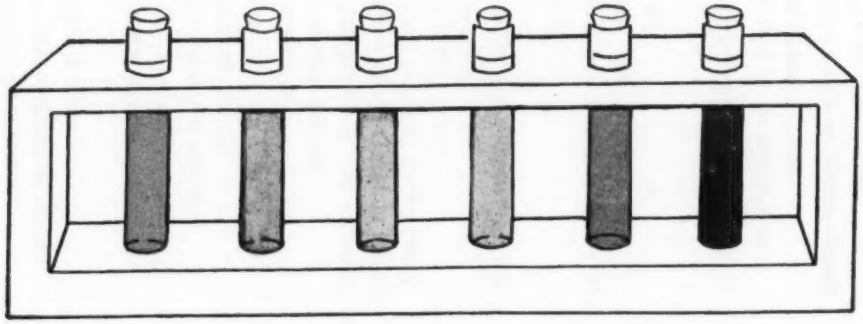
COLOUR STANDARDS

FOR

THE ESTIMATION OF ORGANIC CARBON IN SOIL

Fig. 1



Org. C. in mgm. per 100 grms. of Soil

STUDIES ON THE EMBRYOLOGY OF MICROCHIROPTERA

Part III. The Histological Changes in the Genital Organs and Accessory Reproductive Structures during the Sex-cycle of the Vespertilionid Bat—*Scotophilus wroughtoni* (Thomas)

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1. Introduction

The genital organs and accessory reproductive structures undergo marked histological changes in relation to the sexual rhythm. Heape (1900) gives an excellent summary of the changes which the genital organs undergo during the breeding season. This work focussed considerable attention on this problem and a large volume of information is now available on such changes in the various species of mammals. Some observations have been made on the general sex-cycle of bats but the information is neither detailed nor complete. It has been known for a very long time that the breeding habits of the bats are quite different from those of many other mammals in several important particulars. It was observed by van Beneden (1880) and subsequently confirmed by many authors that bats undergo hibernation during winter, particularly those species which inhabit the temperate and cold climates. It was clearly brought out that in many European and North American bats a long interval of time intervened between copulation and ovulation. The sexual rhythm of bats is therefore unique, and a systematic study of the histological changes in the male and the female gonads and other accessory organs should prove to be of interest and value. The detailed account of Matthews (1937) of two species of British Horse-shoe bats is confined only to the females.

I have already dealt with the general nature of the sex-cycle in *Scotophilus wroughtoni* in my earlier papers (1947 and 1948), and in the present paper it is my purpose to give a fairly detailed account of the histological changes in the gonads and the accessory structures in the females and males of this Vespertilionid bat.

This species is an inhabitant of the geographical tropics. The specimens were collected round about Bangalore, which, unlike other places in Peninsular India, enjoys an equable climate all round the year.

2. Material and Methods

The data of collections have already been given in my previous papers (1947 and 1948). In all 126 females and 29 males were collected all round the year. The gonads and accessory structures, *viz.*, mammary glands in the female, prostates, ampulla of Henle, Cowper's glands in the male were fixed in Bouin's fluid, San Felice's fixative and Carnoy. Sections were cut at 10 μ and stained in Ehrilich's hæmatoxylin and eosin, Delafield hæmatoxylin and eosin, and iron hæmatoxylin. Material fixed in San Felice and Carnoy was stained by the Feulgen technique. Microscopic measurements were taken wherever necessary. The interstitial cells of the testes were measured by counting the total number of interstitial cells in every tenth section of

the testis of every stage and an average of the total number to section was calculated. This average gives a fairly accurate idea of the number of interstitial cells per section. It is however true that the sizes of the sections vary in the different regions of the testis, but this occurs in the testes of all stages; and thus it is possible to obtain a *relative* picture of the abundance or otherwise of interstitial cells. I feel that the conclusions based upon these numbers would not be wrong.

3. General Nature of the Sex Rhythm

Scotophilus wroughtoni breeds once a year, the breeding season is confined to a short period during the middle of March, when copulation occurs and is followed immediately by pregnancy. Young ones are brought forth towards the end of June or early in July. The period of lactation extends up to about the end of August as young ones were not seen attached to the breasts of the mothers in September. This is confirmed by the histological structures of the mammary glands. The young ones have a rapid growth because the specimens collected in September showed no significant size differences and as such classification into juvenile and adult animals was impossible.

The anœstrus period in the female extends up to January when the reproductive activity seems to be stimulated. The specimens collected in February show progressive changes. The œstrus period is very sharply defined as all the female specimens collected on the 22nd of March show early morulæ in their uteri, thus indicating that copulation must have occurred some time in the beginning of the third week of March. Copulation invariably results in pregnancy showing that all the females become sexually mature in their first season before they are one year of age. Pregnancy occurs in both the horns of the uterus and hence they bear two young ones in each litter.

The sexual rhythm in the males is parallel to that of the females, though the males become sexually active a few weeks earlier in the year. From December to March the males are fecund as they show the occurrence of spermatozoa in the genital organs. As the species under consideration is a periodic breeder the seasonal changes in the genital structures of the males are well marked, as in the cases of all the mammals which have a distinct and strictly confined breeding season.

The plan of the male reproductive organs in *Scotophilus wroughtoni* is given in my earlier paper (1948). The male bats have been shown to be quiescent from May to December. Spermatogenetic activity starts in

December, and continues up to copulation after which the regression is very rapid and the specimens collected in April have inactive testes.

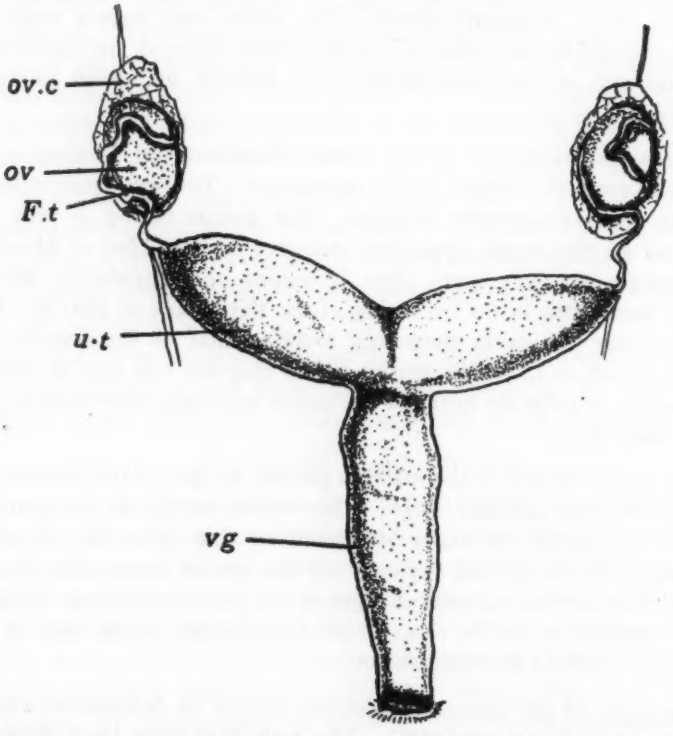
The general sex-cycle may be summarised as below:—

Months	Females	Males
April–July ..	.. Pregnancy	
July–September	.. Lactation	Inactive testes till Dec.
September–January	.. Anœstrus	
January–February	.. Proœstrus	Active testes
March ..	.. Oestrus and copulation	

4. The Histological Changes in the Gonads and the Accessory Structures

A. The Female

(i) *The plan of the reproductive organs* (Text-Fig. 1)



Text-Fig. 1. The female reproductive system of *Scotophilus wroughtoni* (Thomas). *F.t* Fallopian tube ; *ov*, ovary ; *ov.c*, ovarian capsule ; *ut*, uterus ; *vg*, vagina.

The reproductive organs in the female consist of a pair of ovaries which are oval in shape, the Fallopian tube, a symmetrical bicornuate uterus measuring about 1·2 cm. across the two cornua, and a caudally extending vagina measuring about 1·4 cm. in length.

The ovary is elliptical in cross-section with a lengthwise diameter of 1·6 mm. and a breadthwise diameter of 0·5 mm. in specimens collected in præstrus in February. The ovary is completely enclosed inside an ovarian capsule unlike in the British horse-shoe bats (Matthews, 1937). The surface of the ovary is smooth and the follicles do not project out beyond the surface. The ovary is attached to the broad ligament at the hilus at which point the blood vessels enter it. The Fallopian tube takes a circuitous course and emerges on the lateral side and opens into the respective uterine cornu.

The uterus is placed horizontally across the rectum and is held in position by the broad ligament. The vagina is muscular and runs caudally opening into the urinogenital sinus into which also opens the urethra. The vulval opening is transversely oblong.

There are two pectoral mammæ, one on each side, and each with a well developed nipple which becomes particularly obvious during the later part of pregnancy. Pelvic mammæ are totally absent.

(ii) *The ovarian cycle*

In *Scotophilus wroughtoni* both the ovaries are well developed and are functional. Only in one specimen in my collection there was no ovulation in the right ovary while the left ovary showed two coropora lutea. This case is certainly an exceptional one as the right ovary in this specimen did not indicate any sign of degeneration or atrophy.

(*a*) *The Virgin ovary* (Plate II, Fig. 1).—A section of the virgin ovary presents a large number of Graafian follicles in various stages of early development. These follicles are small and the oocytes are often without any covering of follicle cells. The covering, if present, is not more than one layer of cells. These cells are flat and almost form a cellular membrane to the oocyte. The oocytes can be distinguished from the rest of the cells in the ovary by their large size and vesicular nuclei. The stroma of the ovary are made up of loose connective tissue supplied with blood vessels. The developing ova are seen sinking into the medullary area. The largest ovum has a diameter of 37 μ and the largest follicle a diameter of 55 μ in cross section. The number of such primary follicles is very great though each follicle is neither large nor fully formed. The ovum is without a distinct zona membrana.

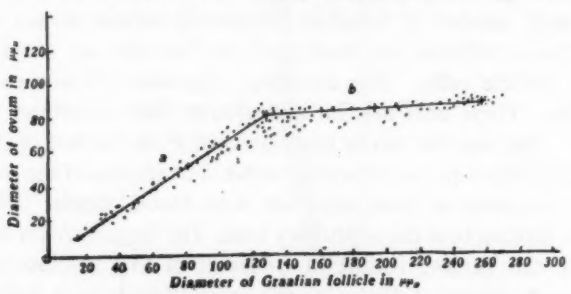
(*b*) *Growth changes in the ovary.*—Just before ovulation during February the ovary shows a faint distinction into cortical and medullary parts. The

tunica albuginia is negligible. There are a number of Graafian follicles at various stages of development in the peripheral part of the ovary. Larger follicles are found more inwards. Some of these are undergoing regression. Amongst these follicles, the ovum that is going to be discharged is easily made out by its large size. The germinal epithelium is still active because the section shows the presence of epithelial nodules with ova and Pfluger's tubes here and there. The ovarian stroma has a large number of blood vessels (Plate II, Fig. 2 and 3).

During pregnancy the growth of the corpus luteum is very rapid and towards the later period of pregnancy, it fills almost the whole of the medullary region. Few Graafian follicles persist at the periphery but they are undergoing regression. The germinal epithelium does not show any indication of ovogenesis.

By September, the corpus luteum will have degenerated and the ovarian epithelium again shows activity. Small Graafian follicles appear at the surface. The medulla is, however, composed of loose connective tissue. By February next the ovary again comes to the proœstrus condition with the formation of large follicles.

(*c*) *Growth of the Graafian follicle.*—The growth of the Graafian follicle takes place in two distinct stages. During the first stage of growth the enlargement of the ovum and the follicle keep a parallel pace until the ovum reaches a maximum size of about 85 μ at which stage the Graafian follicle measures 120 μ in diameter. After the attainment of this size the diameter of the ovum does not increase any further, while the diameter of the Graafian follicle increases by the rapid multiplication of the follicle cells. This simultaneous growth of the ova and the follicle in the first instance, and the growth of the follicle later is clearly indicated by the graph (Text-Fig. 2). The follow-



TEXT-FIG. 2. Graph to indicate the relationship between the growth of the ovum and the growth of the follicle. '*a*' represents the first stage of growth when the increase in the size of the ovum is parallel to that in the follicle, and '*b*' represents the second period of growth when the ovum stops further growth, while the follicle enlarges enormously.

ing equation indicates clearly the relative rate of growth of the ovum and follicle during the two stages mentioned above:—

First stage: $y = 11 + 0{\cdot}6136\ x + 1{\cdot}5921$

Second stage: $y = 82 + 0{\cdot}0456\ x + 76{\cdot}1584$

where y represents the size of the ovum and x represents the size of the follicle. It is during the second period of growth that the antrum begins to develop. The beginning of the formation of the antrum in the form of intercellular spaces was first recognised in a Graafian follicle which was 171 μ in diameter.

(*d*) *Development of the Graafian follicle.*—I have reasons to believe that in *Scotophilus wroughtoni* the germinal epithelium is an active agent in producing ova in the post-pubertal life. In a full term foetus the germinal epithelium of the ovary shows here and there certain cells which are slightly larger in size and spheroidal in shape, while the rest of the cells are flattened. These are referred to as the epithelial nodules by some authors. These are unmistakably the oocytes (Text-Fig. 3; Pl. II, Fig. 3). These oocytes appear to be only modified cells of the germinal epithelium. These early oocytes migrate inwards and come to lie in the cortical part of the ovary. The oocyte

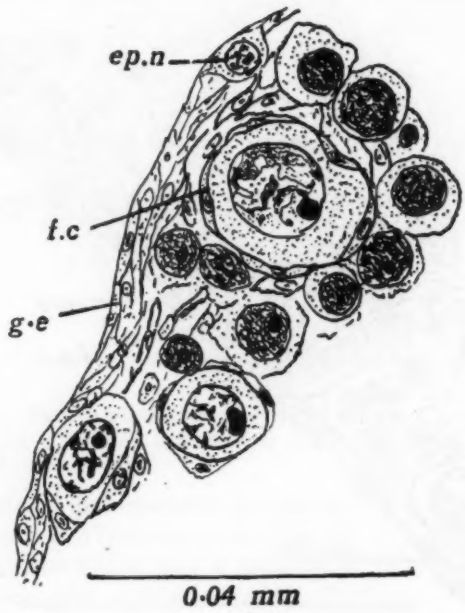


FIG. 3

at this stage has a diameter of 10 to 14 μ and is surrounded by a layer of flattened cells (Text-Fig. 4). The nucleus of the oocyte is large and is placed centrally, and has one or two darkly staining chromatin granules. A clear zona pelucida does not occur, but there is only a cytoplasmic border for the ovum (Text-Fig. 4 *a*).

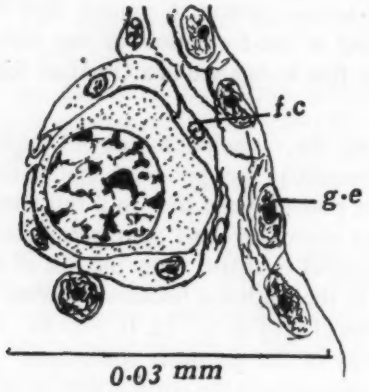


FIG. 4.

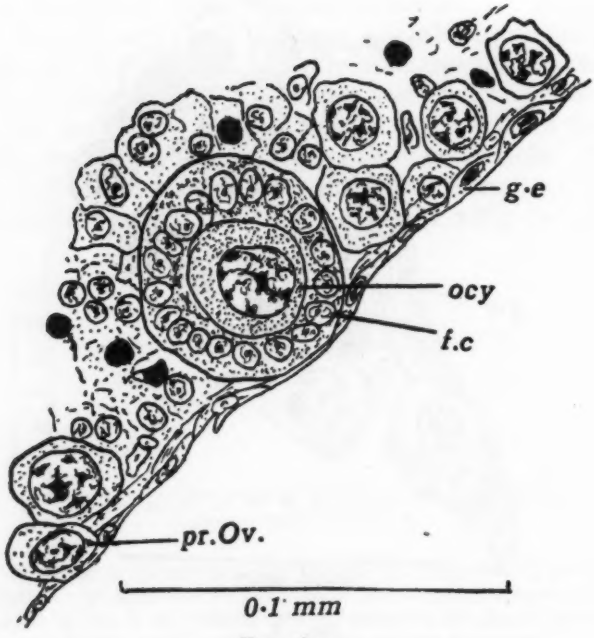
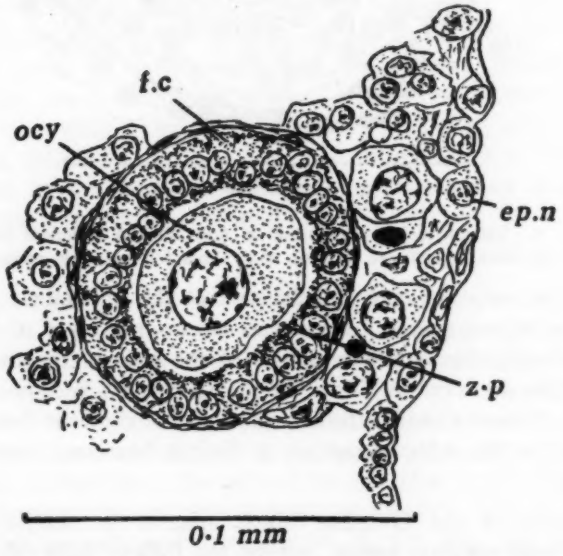


FIG. 4 *a*

TEXT-FIGS. 3–4 *a*.—Fig. 3. A camera lucida drawing of a portion of a section of the ovary of a specimen collected in February. Note the primary oocytes being differentiated from the germinal epithelium. *ep.n*, epithelial nodules; *f.c*, follicular cells; *g.e*, germinal epithelium. Fig. 4. Camera lucida drawing of an early Graafian follicle. Note the flattened cells surrounding the ovum. *f.c*, follicle cells; *g.e*, germinal epithelium. Fig. 4 *a*. Camera lucida drawing of a portion of a section of the active ovary of February specimen showing the differentiation of the oocytes from the germinal epithelium. *f.c*, follicular cells; *g.e*, germinal epithelium; *o.cy*, oocyte; *pr.ov* primordial ovum.

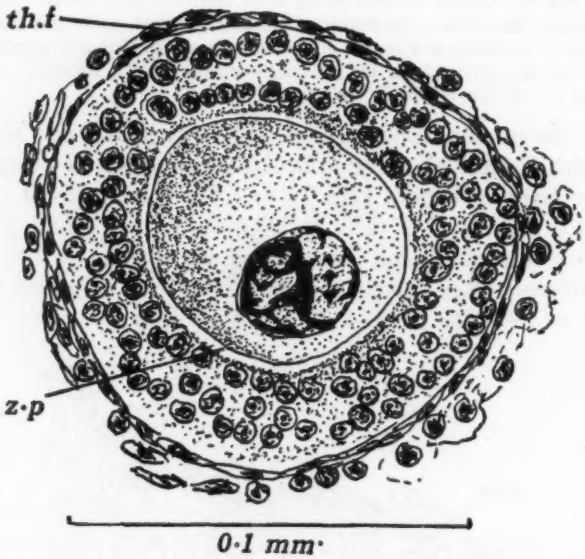
Inside the primary follicle the ovum grows in size and the nucleus becomes gradually eccentric. The follicular covering will be completely formed as a single layer of cubical cells. The further enlargement of the follicle cells and the migration of their nuclei towards their distal portions result in producing a protoplasmic zona round the ovum. A fully formed unilaminar primary Graafian follicle has a diameter of 63 μ and the ovum has a diameter of 39 μ (Text-Fig. 5; Pl. II, Fig.4).



TEXT-FIG. 5. Camera lucida drawing of a fully formed unilaminar Grafiaan follicle. The theca folliculi is not fully differentiated. *ep.n*, epithelial nodules; *f.c*, follicular cell; *ocy*, oocyte; *z.p*, zona pellucida.

As a result of the repreated divisions of the follicular cells, the follicle becomes bilaminar (Text-Fig. 6; Pl. III, Fig. 5). When fully formed the bilaminar Graafian follicle has a diameter of 143 μ. From this stage onwards the follicle cells proliferate rapidly and form a many-layered covering for the ovum. These cells are densely and uniformly arranged. By now

the ovum will have reached the maximum size beyond which it does not increase markedly in size.



TEXT-FIG. 6. Camera lucida drawing of a fully formed bilaminar follicle. The zona pellucida and the theca folliculi are formed. *th.f*, theca folliculi; *z.p*, zona pellucida.

In the primary Graafian follicle even at the unilaminar stage a theca folliculi may be recognised. It consists of a single layer of flattened cells formed probably by a differentiation of the cells of the ovarian stroma. As the follicles become multi-layered the theca also becomes two layered. In the fully formed Graafian follicle the theca is three or four layered and is composed of flat cells placed on a distinct basement membrane (Plate III, Fig. 6).

The antra of the Graafian follicle arise at the multilayered stage as chinks or inter-cellular spaces amidst the follicle cells (Pl. IV, Fig. 7). Finally the various spaces coalesce and form the large antrum cavity. The Graafian follicle when fully formed has a diameter of 277 μ and the ovum has a diameter of 93 μ.

Though a large number of Graafian follicles commence development most of them undergo regression at some stage or other, only one becoming successful in undergoing complete development and rupture. The degeneration of the Graafian follicle usually starts with the ovum, because in most of the degenerating follicles the follicle cells were intact (Pl. IV, Fig. 8).

In the earlier stages of pregnancy the ovary presents a large number of Graafian follicles in various stages of degeneration and regression. The degenerating follicles are characterised by the fragmentation of the nuclei of the ova and the highly pycnotic nature of the nuclei of the cells of the follicle. It is also held by some authors that some of the peripheral cells of the follicle which are abutting against the developing corpus luteum may undergo luteination.

(*e*) *The corpus luteum.*—After ovulation the diameter of the corpus luteum is 390 μ, which is far greater than the diameter of the largest Graafian follicle. The development of the corpus luteum after this stage is very rapid. The central part of the corpus luteum is, in the early stages, occupied by extravasated blood which continues to be present till the embryo has attained the stage of the unilaminar blastocyst (Pl. V, Fig. 9). By the time the blastocyst has implanted the central cavity of the corpus luteum will have been obliterated by the ingrowth of luteal cells. As pregnancy advances the corpus luteum also enlarges. Thus on the 7th of June when the embryo advanced far beyond half-term the corpus luteum occupied almost the entire ovary except for a small peripheral cortical area. The corpus luteum never projects beyond the surface of the ovary as in the British horse-shoe bats (Mathews, 1937). After parturition which occurs in early July the corpus luteum begins to disappear and the ovary in September shows the normal anœstrous condition.

(iii) *The Fallopian tube*

The fallopian tube during the breeding season does not undergo very marked changes. The tube can be distinguished into two regions depending upon the histological structure—an ovarian part possessing predominantly glandular cells, and a uterine part possessing predominantly cilitated cells. The ovarian part of the tube shows complex epithelial folds while in the uterin part the epithelium is essentially of the lining type with few folds. In the inactive animal, in September, the cells of the epithelial lining of the ovarian part of the fallopian tube are closely crowded together, cubical and small. The lumen itself is small and irregular (Pl. V, Fig. 10), but during the active period in February, the epithelial folds enlarge and the nuclei occur towards the bases of the columnar cells. The cilia become very clear and the epithelial lining begins to be thrown into folds. There is a general increase in the diameter of the lumen due to a definite swelling of the tube as a whole. The glandular or goblet cells also become clearly visible. The lumen often shows the presence of secretion, and also cellular debris probably derived by the break down of some of the epithelial cells due to over-hypertrophy (Pl. V, Fig. 11).

During pregnancy the Fallopian tube reverts to the anœstrous condition. This was noticed in specimens collected in late April, when the uterus was gravid and contained fairly advanced embryos. The epithelial cells become cubical and the nuclei fill up the cells.

(iv) *The Uterus*

Detailed histological changes in the uterus will be described in the next part which will deal with the early development and placentation. Only a brief account is included here.

(*a*) *Anœstrus uterus.*—The uterus has a small lumen with very few small straight unbranched glands, which often do not have a lumen. The uterine lumen is elongated in the dorso-ventral axis being narrow sideways. A distinct implantation chamber as occurs in some mammals cannot be recognised, except for a slight widening of the antimesometrial part of the lumen. The uterine epithelium is continuous and the cells are columnar, having a height of 8 μ to 12 μ (Pl. VI, Fig. 12).

(*b*) *Changes in the uterus.*—At the onset of sexual activity there is a general swelling of the uterus and an increase in its vascularisation. The endometrial tissue has become less dense. The cells of the uterine epithelium increase in height to 18 μ to 20 μ, and the glands become enlarged. These glands are sometimes lobed and may extend up to the outer border of the endometrium. All the glands have distinct lumina. At the height of sexual activity the uterine lumen also widens. The glands increase in number and secretory products and cell debris are commonly seen inside the lumen of the uterus. The uterine epithelium is ruptured here and there due to conjestion of blood in the subepithelial region; and often blood corpuscles are visible in the uterine lumen (Pl. VI, Fig. 13). No sperms were seen in the uterine lumen of specimens collected on 10th February indicating thereby that copulation had not occurred.

(v) *The Vagina*

In the Anœstrous condition the vagina is a muscular organ with a simple lumen which is transversely oblong, the vaginal epithelium is made up of three or four layers of squamous cells of which the deepest layer alone is cubical while the superficial cells are flattened (Pl. VII, Fig. 14).

On the onset of reproductive activity the cornification of the vagina increases and at the height of sexual activity a great amount of desquammation occurs resulting in the rupture of the cells which are seen to be filling the vaginal lumen. The lumen itself becomes large and shows the presence

of folds. The vaginal walls show great hypertrophy and vascularisation (Pl. VII, Fig. 15).

During pregnancy the vaginal epithelium is reduced in thickness, the lumen is large and the folds of the epithelium so characteristic of the œstrus condition are lost. The epithelium is made up of three or four layers of cells. The lumen is clear and does not contain any degeneration products.

(vi) *The mammary glands*

The non-lactating mammary gland consists of a number of lactiferous tubules which are separated by a dense connective tissue which has a fibrous appearance. Each lobule is in itself made up of a number of lactiferous glands having a common duct. The glands are separated by a loose connective tissue. Individual glands are small and have small lumina which are empty. The loose connective tissue is said to have a functional significance " in providing an easily distensible medium for the hypertrophy of the epithelial portions of the organ during pregnancy and lactation " (Maximow and Bloom, 1947). The interlobular area is rich in adipose tissue (Pl. VIII, Fig. 16).

During pregnancy the mammary glands undergo profound changes. The first change noticeable is the enormous hypertrophy of the secretory alveoli and the increase in size of the ducts. The interlobular connective tissue is reduced and the adipose tissue becomes insignificant. The secretory portions show a number of lactiferous pouches in which milk is secreted (Pl. VIII, Fig. 17).

The glands undergo very rapid regression so that the mammary glands of specimens collected in September were very small without distinct nipples. Their histology resembled the glands in the anœstrus specimens.

B. The Male

(i) *The Interstitial Tissue*

The following Table gives the average number of interstitial cells per section of the testis of specimens collected during the several months of the year. I have already mentioned the method I have employed in counting these, and I feel that it gives a fairly accurate picture.

It is apparent from the Table that the largest number of interstitial cells occur in the testes of the specimens in which the activity of the seminiferous tubules is at its highest. It is particularly interesting to note that there is a progressive increase in the number of interstitial cells from 1100

TABLE 1 Table showing the number of Interstitial cells in the various months in *Scotophilus wroughtoni* (Thomas)

Month	Date	Index No.	Average weight of two testes in milligrammes	No. Interstitial cells per sec.
January ..	8-1-47	123	143·5	1527
"	"	129	130·0	1306
February ..	10-2-46	32 (vi)	151·0	1877
"	"	32 (vii)	139·5	1760
March ..	22-3-46	33 (c)	49·5	842
"	"	33 (d)	52·5	876
April ..	1-4-46	45	46·0	789
"	29-4-46	88	35·5	762
May ..	7-5-46	101	36·0	718
"	17-5-45	27	31·0	615
June ..	7-6-46	108 (a)	..	582
"	"	111 (f)	..	642
July ..	No collections		..	..
August				
September ..	18-9-46	113 (a)	39·5	620
October ..	20-10-45	30 (a)	50·0	816
November ..	8-11-45	31 (c)	48·5	794
"	"	31 (d)	46·0	842
December ..	7-12-46	116 (a)	93·0	980
"	"	118 (b)	109·5	1100

to 1877 during the months of December, January and February. Text-Fig. 7 is a graph to indicate the changes in the number of interstitial cells in the

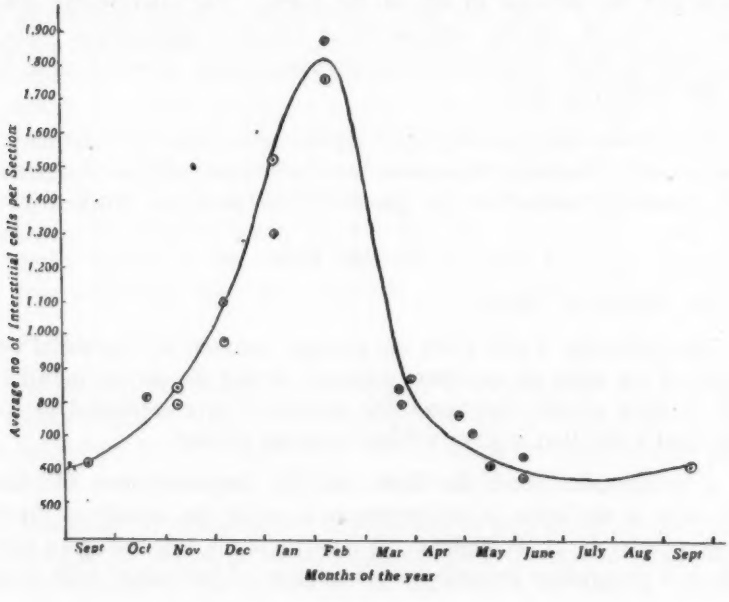


FIG. 7

several months of the year. On February 10th when the Leydig cells are most numerous in the testes, copulation had not occurred as shown by the examination of the female specimens. The seminiferous tubules are very active and swarms of spermatozoa can be seen in the tubules of the epididymis as shown in my earlier paper (1948). Text-Fig. 8 is a graph to indicate the relationship of the number of interstitial cells to the weight of the testis. Taking the weight of the testis as an indication of spermatogenetic activity it clearly indicates that the number of interstitial cells is greatest at the period when spermatogenetic activity is also at its highest.

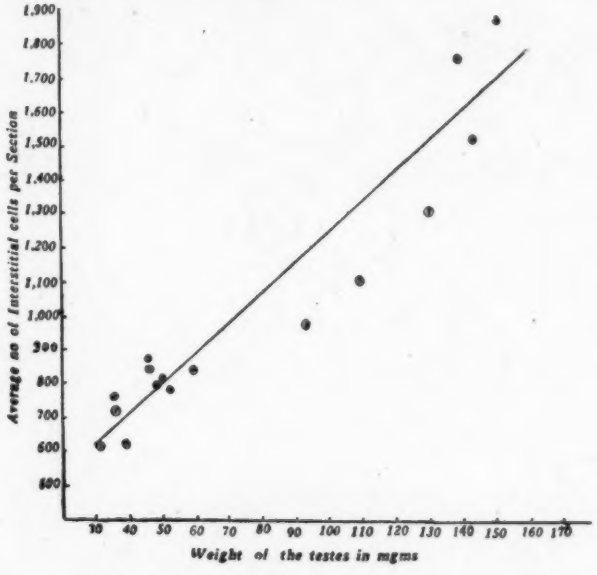


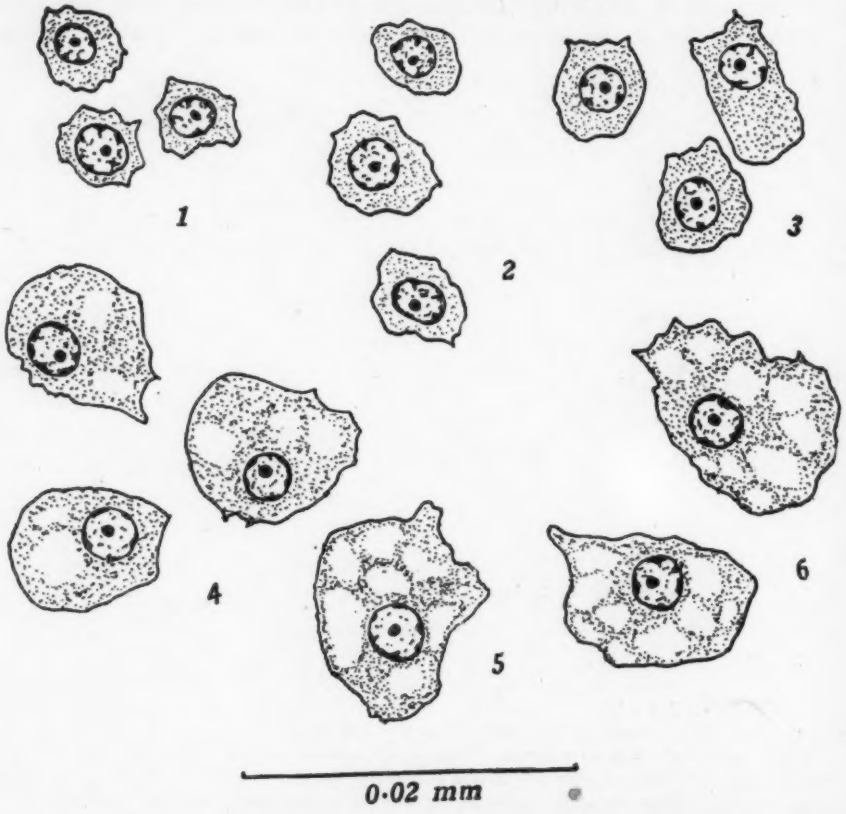
FIG. 8.

TEXT-FIGS. 7–8.—Fig. 7. Graph to indicate the number of interstitial cells per section of the testis in the several months of the year. The number of interstitial cells increases suddenly from December to February. Fig. 8. Graph to indicate the relationship between the number of interstitial cells per section and the weight of the testis. Graph is a rising curve showing that as the weight increases the number of interstitial cells also increases.

After copulation which occurs somewhere in the middle of March the males and the females live separately as indicated by the collection data given in my previous papers (1947 and 1948). There is a decrease in the number of interstitial cells as shown by Table I and Text Fig. 7, and this decrease goes on progressively till September. The smallest number of interstitial cells were counted in testes of specimens collected between May

and September. The number after September again increases gradually till December, after which there is a sudden increase.

Text-Fig. 9 is a series of camera lucida drawings of the Leydig cells at the same magnification during the different months of the year. The figures clearly indicate that the sizes of the cells also increase during the breeding season. The Leydig cells in February are clearly seen to be nearly three or four times the sizes of the Leydig cells in May to September.



TEXT-FIG. 9. Camera lucida drawings of interstitial cells (Leydig cells) in the several months of the year. All the cells are drawn at the same magnification. The size of the interstitial cell in February is nearly three or four times the size of the cell in May. Note also the occurrence of vacuoles in the active cells in December, January and February.

Thus both the number and size of the Leydig cells are maximum at the height of spermatogenetic activity of the testis. The Leydig cells are most active when spermatogenetic process is also active.

Another point of histological interest is that there seems to be a cyclical phenomenon in the intertubular connective tissue. When the spermatogenetic activity is going on and the Leydig cells are most numerous the intertubular connective tissue is found to be very scanty, while in the specimens collected in May when the testis is quiescent, Leydig cells are fewer, the intertubular area is practically completely occupied by connective tissue cells. Furthermore, I have not been able to see a single nucleus of an interstitial cell preparing for division nor the occurrence of spindles inside these cells. The nuclei are invariably vesicular with the chromatin being disposed towards the periphery of the nucleus and with one or two nucleoli. It is however normal to find 6 to 20 Leydig cells occurring in clusters as in the Wood Chuck (Rasmussen, 1917), but the complete absence of any cell division stages makes it quite certain that this clustering is only an arbitrary crowding and cannot be the result of divisions of a single parent cell. This fact taken along with the fact that the connective tissue has an alternating cycle with interstitial tissue makes it probable that these two are inter-convertible tissues.

(ii) *Changes in the ampulla of Henle*

During the quiescent period the ampulla of Henle shows a large amount of connective tissue between the glandular parts. The lumina of the tubules are small and clear and the epithelium is cuboidal (Pl. VIII, Fig. 18). But during February there is a great hypertrophy of the glandular part. The connective tissue is much reduced and the lumina become enlarged and filled with secretion (Pl. VIII, Fig. 19).

(iii) *The changes in the prostates*

The changes in the prostate glands are parallel to the changes in the ampulla of Henle. The quiescent prostate of May or September shows a large number of glandular tubules which are separated by a great amount of connective tissue. The glandular tubules have an epithelium devoid of basement membrane and as such the tubules appear as small spaces in the connective tissue (Pl. IX, Fig. 20). But on the onset of activity the tubules become enlarged and the epithelium becomes better defined and distinct from the connective tissue which is reduced to mere strands. The glands are filled with secretion (Pl. IX, Fig. 21).

(iv) *The Cowper's glands*

The Cowper's glands or the Bulbo-urethral glands also show marked cyclical changes during the different seasons of the year, correlated with sexual activity of the animal. The lumina of the glands increase in size and

they are filled with secretion during the breeding season while the alveoli are small and empty during the non-breeding seasons (Pl. IX, Fig. 22).

5. Discussion

(*A*) *The reproductive organs*

The reproductive organs of Chiroptera are characterised by their bilateral asymmetry, and symmetrical condition is rare. It is usually the right side that becomes more developed than the left (Wood, 1917). Wood Jones (1917) states that in Vespertilionidæ, the left cornu has practically disappeared and the uterus becomes a simple corpus uteri, showing traces of the presence of the cornua at the angles. Matthews (1942) states, "One of the most interesting characters of the female genitalia of the Microchiroptera is the bilateral asymmetry, which occurs in varying degrees of intensity..... Most bats except those of the family Phyllostomatidæ have a bicornuate uterus, but nearly always bring forth only one young at a birth; consequently as a rule only one uterine cornu is occupied by pregnancy. It has been found in very many species of different families that there is a constant tendency for the right side of the genitalia to be the functional one". He has also observed in *Rhinolophus hipposideros minutus* that the left ovary appears to be degenerate never producing any ova, the pregnancy being always on the right side (Matthews, 1937). However, in *Scotophilus wroughtoni* the genitalia are perfectly symmetrically developed on the two sides, there being two functional ovaries and two distinct uterine cornua, each bearing a fœtus during pregnancy.

(*B*) *The growth of the Graafian Follicle*

Most of the authors working on the sex cycle of mammals have shown that the development of the follicles takes place in two stages. During the first stage the ovum and the follicle cells grow simultaneously until the ovum attains a maximum size. During the second stage only the follicle increases while the ovum does not undergo any more growth. This results in the production of a Graafian follicle with a many layered follicular covering for the ovum. This fact has been clearly demonstrated in many mammals such as mouse (Brambell, 1928); rat, ferret, and pig (Parkes, 1931); Grey squirrel (Deanesly and Parkes, 1933); Hedgehog (Deanesly, 1934); Stoat (Deanesly, 1935); the common shrew (Brambell, 1935); Bank vole (Brambell and Rowlands, 1936); the British horse-shoe bat (Matthews, 1937); *Myotis lucifugus lucifugus* (Wimsatt, 1944). In *Scotophilus wroughtoni* too, as in all these mammals, the growth of the follicle takes place in two stages.

Some authors have suggested a physiological relationship between the growth of the ovum and the growth of the follicle cells. Brambell's (1928)

view is that the growth of the ovum during early stages is due to the nutritive activity of the ovum, which, after the ovum has attained the maximum size, and has accumulated the largest amount of material, allows the growth of the follicle cells, and increase in their numbers. This view has been recently supported by Wimsatt (1944).

(*C*) *Post-natal origin of ova*

There are two mutually exclusive views on the potentiality of the germinal epithelium for producing ova in the post-natal life of the animals. One view is that all definitive ova are already differentiated from the germinal epithelium at birth and their further development in life consists in growth and nuclear changes. This view is held by Felix (1912) and Hamel (1935). Felix supported this view on the ground that the germinal epithelium does not show nuclear divisions in the adult and that the tunica albuginia, after it is formed, acts as an effective barrier for the migration of any ova through it into the cortical part of the ovary. The second view is that the germinal epithelium produces ova in the post-natal life as long as the sex period lasts. Thus Hargitt (1930 *a* and *b*) has proved in the rat that the primary ova which are produced in large numbers in the embryonic condition all degenerate sooner or later, and the definitive ova are produced afresh in the germinal epithelium in the adult. He categorically denies the possibility of the primary ova persisting in a dormant condition for such a long time. He agrees with the conclusions of Papanicolauo (1924) that the follicle cannot remain dormant but must undergo either development or degeneration. He believes that "No embryonic germ cell could persist until sexual maturity" Edgar Allen (1923) also came to the same conclusion in the mouse. He stated that " The establishment of the continuance of ovogenesis during sexual maturity, eliminating the supposed long dormant period in the ovarian life of the ovum, makes the process more nearly analogous to spermatogenesis." Mossman (1937) has shown in the Pocket gopher that the germinal epithelium retains its ovogenetic potentiality in the adult. Pliske (1938) has recorded in the Thirteen Striped ground squirrel that the germinal epithelium plays a major role in the post-pubertal ovogenesis of the mammalian ovary. Mary J. Guthrie and Katherine Jeffers (1938) working on bat ovaries observe that "The ovaries of *Myotis lucifugus lucifugus* and *M. greisescens* indicate that oocytes are formed during sexual maturity, although mitosis has not been observed."....."It is clear that the cells of the tunica albuginea are pushed out of their alignment by growing cells in the germinal epithelium and offer no barrier to their movement in the deeper positions." The occurrence of epithelial nodules and the transitional stages of the cells of the germinal epithelium towards the formation of the ova in *Scotophilus*

wroughtoni strongly support the latter view that the germinal epithelium in the adult is the source of the definitive ova; the primary ova produced in the embryonic stages undergo degeneration before sexual maturity. My observations support the view of Edgar Allen (1923) that "A cyclical proliferation of the germinal epithelium gives rise to new addition of young ova to the cortex of the adult ovary at each normal œstrous period."

(*D*) *Interstitial tissue of the testis.*—

No accessory reproductive structure in the male presents a greater divergence of opinion as the interstitial cells of the testis. The divergence relates to four aspects: the relation of the number and size of these cells to spermatogenetic activity; the origin of the interstitial cells in the post-natal life; the periodic degeneration of these cells; and the functions of these cells.

(i) *The relationship of the number and size of the interstitial cells to spermatogenetic activity.*—A perusal of the relevant literature reveals that there is considerable difference of opinion as regards the number and size of the interstitial cells during the different stages of sperm producing activity of the testis. Some authors hold the view that in periodically breeding animals the interstitial tissue is most active when the seminiferous tubules are quiescent, and it is least active when the spermatogenetic activity is at its highest. Champy (1909) states that in *Rana esculenta* when the seminiferous tubules are most active, in July, the interstitial tissue is at its minimum. An exactly similar observation is made by Bissonnette (1930) in the British Starling where there was the occurrence of the maximum number of interstitial cells during the non-spermatogenetic season and a minimum number when spermatogenesis is at its peak.

Many authors working on mammalian sex-cycle hold the same view. Regaud (1904) recorded in the Mole (*Talpa europea*) that the interstitial cells are abundant in the quiescent testis, while few in the active testis. Tandler and Grosz (1911) working on the mole observe—" When the spermatogenesis is at its highest, the interstitial cells are at their lowest, and *vice versa*" (Quoted from Rasmussen, 1917, page 482). This view is largely supported by Rasmussen (1917). His graph on page 501 indicates that there is no parallel development of the interstitial cells with the spermatogenetic activity. " The interstitial cells suddenly undergo retrogression while spermatogenesis goes on uninterrupted. The interstitial cells remain apparently inactive and greately reduced for many months while spermatogenessis goes on progressively." Roland E. Miller (1939) states that in *Myotis lucifugus lucifugus* and *M. greisescens* "The maximum size of the interstitial cells is attained

in July, thereby preceeding the greatest activity of the seminiferous tubules, namely meiotic divisions," but " with the reduction of the size of the testis, correlated with the retrogression of the seminiferous tubules, the relative numbers of the interstitial cells appear to increase; the interstitial tissue occupies a greater volume of the testis than before." Oslund (1928) has also collected data relating to a number of mammals to support this view.

Exactly opposite are the observations of some other workers in certain other animals. Friedmann (1898) has shown in the frogs, *Rana fusca*, *Rana viridis*, and *Hyla arborea*, and in the toad *Bufo vulgaris* and subsequently confirmed by Mazetti (1911) in *Rana fusca*, and *R. viridis*, that the seasonal changes in the interstitial tissue run parallel to the seasonal activity of the seminiferous tubules. There is an increase in the interstitial cells in autumn when spermatogenesis progresses, while they remain minimal when spermatogenetic activity ceases. Amongst the mammals too a similar phenomenon has been observed by many workers. Hansemann (1895) has observed in the Marmot that " The testis of the hibernating animals in which there is no spermatogenesis contains practically no interstitial cells, there being only a few spindle-shaped cells between the tubules. After the animal has been awake, however, and spermatogenesis is going on the interstitial cells are very numerous " (Rasmussen, 1917, page 478). Marshall (1911) supports this view by observations on the hedge hog, where he noted a simultaneous increase in the interstitial tissue and spermatogenetic activity in the rutting season during April and May.

Working on *Apodemus flavicollis* and *A. sylvaticus* Groom (1933) has shown that " There is an absolute increase in the number and size of the interstitial cells in the breeding season." He suggests that the difference of opinion is caused by a confusion amongst the workers, because their study was a correlation of the growth changes in the seminiferous tubules and those of the interstitial tissue. " In birds as well as in mammals, dealing with relative rather than absolute number of interstitial cells appears to have led to this confusion " (Groom, 1933).

Working on Stoat, Deanesly (1935) has noted that the interstitial cells are grately enlarged in size in March, April and May. This is the season when the testis is most active in producing spermatozoa. In the male Weasel, Margaret Hill (1939) noted that " There is a definite cycle in the interstitial cells; they are large during the breeding season, the average cell being 180 μ to 250 μ, and are small and crowded together in the non-breeding seasons, when the average cell is 80 μ to 90 μ". She does not, however, mention any numerical changes. In *Pteropus geddei* Groom (1940) discovered some very interesting features. He counted the largest number of interstitial cells

at the period of copulation which occurs about February, and this number decreases very much during July and August, when the male bats live in isolation.

A very important distinction was brought by Courrier (1927), Miller (1938), and Hooker (1944) who believe that the functional activity of the interstitial tissue as a source of internal secretion is not indicated so much by the numbers of the cells as much as by their cytological structure. Hooker (1944) has shown that in the bull " A mere increase in the number of interstitial cells was not accompanied by a great increase in androgen." He further states " As the number of Leydig cells with vacuoles increased, and as the degree of vacuolation in the individual cells increased, the androgen content of the testis exhibited an increase of comparable proportions." So " The cytology appears to be decidedly a more important factor than number of cells in the secretion of androgen."

In *Scotophilus wroughtoni* there is an increase in the absolute number of interstitial cells and also an increase in the size of the individual cells correlated with the increase in the spermatogenetic activity. This has been clearly indicated in Table I and Text-Fig. 9 respectively. In this respect *Scotophilus wroughtoni* agrees with the two species of *Apodemus* and *Pteropus geddei* and with the view expressed by Groom (1933 and 1940) by observations on these animals.

(ii) The *origin of interstitial cells in the post-natal life*.—In the embryo the interstitial cells have been shown by many authors to arise by a direct transformation of the mesenchymal cells. This is the view of Whitehead (1904) working on the pig embryo, Allen (1904) working on the pig and rabit, Bascom (1923) working on the cattle and Hooker (1944) working on the bull. But the origin and the fate of the interstitial cells in such mammals which show a periodic increase in their numbers is a matter of controversy, because of the fact that most of the authors have not been able to record a complete process of cell division in these interstitial cells. Rasmussen (1917) however, recorded the occurrence of binucleate and multinucleate clusters of interstitial cells and concluded that " There is cell division and that each group of cells represents the daughter cells of a single parent cell." He has not seen actual mitotic figures. But certain other workers like " Van Hansemann (1895), Reinke (1896), Van Lenhossek (1897) and Pick (1905) have reported mitotic figures in human material including adult." (Rasmussen, 1917, page 491).

Thus due to the complete lack of systematic observations regarding the mitotic divisions in the interstitial cells most authors arrived at the

conclusion that the interstitial cells are modified connective tissue cells of the intertubular region of the testis. Allen (1904) in the case of the testis of pig and rabbit maintains this view. This theory of the connective tissue origin of the interstitial cells in the adult is also supported by Whitehead (1904) in pig, Kingsbury (1914) in the cat, and Bissonnette and Chapnick (1930) in the European Starling. A confirmation of this view is furnished by Bascom (1923) in cattle and Hooker (1944) in the bull as they have shown the intermediate stages in the transformation of the connective tissue cells into Leydig cells.

In *Scotophilus wroughtoni* I did not see a single interstitial cell in division. All nuclei were large and vesicular without any preparatory changes towards mitosis. It is normal to find clusters of Leydig cells, but the clusters cannot be taken as being derived by divisions of a single parent interstitial cell as was presumed by Rasmussen (1917). This clustering is only a crowding and has no significance whatsoever. The interesting fact is that while the inactive testis has a large amount of intertubular connective tissue, the active one shows a reduction in the connective tissue cells and an increase in the number of Leydig cells. Unless this is a mere coincidence the view that connective tissue cells do get transformed into Leydig cells does not seem to be inaccurate. This assumption, at any rate, would explain the puzzling fact of the periodic increase in the number of interstitial cells in the sex-cycle of the mammals. The problem of the origin of the Leydig cells in the post-natal life bears solution if it is assumed that periodically the connective tissue cells become active and get transformed into Leydig cells.

(iii) *The periodic degeneration of the interstitial tissue.*—Rasmussen (1917) believed that the Leydig cells after a period of activity get transformed into connective tissue cells. But Hooker (1944) states that though the Leydig cells may arise by a direct transformation of the fibroblasts and even lymphocytes, they cannot revert back to the condition of the connective tissue cells once they are formed, but they invariably undergo degeneration. In *Scotophilus wroughtoni* I have not been able to see any disintegrating Leydig cells, but all the evidence points to the conclusion that these Leydig cells can revert back to the connective tissue cells, because correlated with the reduction in the activity of the seminiferous tubules and the reduction of the Leydig cells the connective tissue cells increase in number. It is highly probable therefore that periodically the connective tissue cells assume the form of Leydig cells—for reasons still quite obscure—and after a short period they revert to their original condition.

(iv) *The functions of the Leydig cells.*—These cells are usually considered to be the possible producers of sex harmone. But a number of authors deny that these cells have anything to do with the production of sex harmone. Kingsbury (1914), Blair Bell (1916), and Rasmussen (1917) are sceptical about the interstitial cells being the seat of hormone production. Recently Hooker (1944) has stated that the manifestation of the secondary sexual characters in the bull at puberty is not correlated with any change in the number of character of the Leydig cells. The testicular hormone, according to him, is not responsible for producing great somatic changes as are observed at puberty, because he does not find any change in the androgen content of the testis in the bull at puberty. He has shown that the Leydig cells produce androgen (he does not exclude the possibility of the sertoli cells producing androgen), but androgen, according to him, is not responsible for changes at puberty.

But a large amount of experimental work has accumulated to show that even in sterile animals, if the Leydig cells are in tact, the sex desire of the animal is maintained. Thus the cryptorchid testis is supposed to produce as much sex hormone as the normal testis. Hence Bascom (1923) concluded that the interstitial cells are the seat of sex hormone. Maximow and Bloom (1947) regard the Leydig cells as being responsible for the maintenance and manifestation of secondary sexual characters.

The definition of the functions of the Leydig cells becomes still more difficult in the case of animals which show a periodic increase and decrease in their sizes and numbers, for in certain animals they seem to be most active in the non-breeding seasons while in other animals they are most active during the breeding seasons. They thus behave differently in different animals.

Working on *Pteropus geddei*, Groom (1940) has recorded a curious correlation between the period of co-habitation of the males and the females and the greatest abundance of interstitial tissue. After copulation the males and the females do not live in the same colony, but live in separate colonies. But the interstitial tissue though reduced has not reached its minimum. But when the interstitial cells are reduced to the minimum the male begins to live in isolation. He therefore finds a relation between the activity of the Leydig cells and the social behaviour of the bats and concludes that " The male hormone may to some extent control the purely social behaviour of the bats." The collection data of *Scotophilus wroughtoni* (Gopalakrishna 1947, 1948) points to a similar condition. After March though a large number of females were collected, it was very difficult to collect the males,

indicating thereby that the males and the females do not live together after copulation. Thus whatever may be the manifold functions of the interstitial cells, in *Scotophilus wroughtoni* these cells most probably induce the copulatory stimulus, after which they undergo rapid regression resulting in the separation of the males and the females from the common colony.

(*E*) *The Ampulla of Henle*

The homology of the ampulla of Henle in the reproductive tract of the males of some species of microchiroptera is not fully established. Matthews (1942) records a curious fact in some of the South African bats that wherever the ampulla of Henle occurs, the seminal vesicles are absent. Thus it is possible that the ampulla of Henle may be homologous to the seminal vesicles. This is further borne out by the fact that the structure of the ampulla of Henle closely resembles the structure of the seminal vesicles.

6. Summary

1. The study is based on an examination of 126 females and 29 males collected all round the year.

2. A general sketch of the sex-cycle has been given. Œestrus and copulation have been shown to occur in the middle of March. The males become physiologically active a few weeks earlier than the females.

3. The female genitalia is peculiar in being bilaterally asymmetrical, structurally and functionally.

4. The growth of the Graafian follicle takes place in two stages—during the first stage there is a simultaneous growth of the ovum and the follicle, and in the second stage the follicle alone enlarges.

5. The germinal epithelium produces ova in the adult.

6. The fallopian tube, the uterus, and the vagina also show cyclical histological changes.

7. There is an increase in the number and size of the interstitial cells during the sexual activity, *i.e.*, from December to March.

8. It is hinted that the interstitial cells arise in the adult by a direct transformation of the intertubular connective tissue cells of the testis.

9. After a period of activity the interstitial cells probably revert back to the condition of connective tissue cells.

10. The activity of the interstitial cells probably stimulate copulation and control the social behaviour of the bat under study.

11. The accessory organs in the male all undergo hyperstrophy and produce copious amount of secretion during the breeding season.

12. The ampulla of Henle is probably homologous to the seminal vesicles.

7. Acknowledgement

I am deeply indebted to Prof. M. A. Moghe, Head of the Department of Zoology, College of Science, Nagpur, for the very valuable guidance and help which he has given me throughout my work. My thanks are also due to Mr. P. A. Ramakrishna Iyer, Central College, Bangalore, for the initial training and technical guidance during the early stages of my work.

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Explanation of Plates

(All are Photomicrographs)

List of abbreviations used.—*an*, antrum ; *at*, adipose tissue ; *c.l*, corpus luteum ; *d.c*, desquammated cells ; *ep.n*, epithelial nodule ; *f.c*, follicle cell ; *h* hilus ; *ov.c*, ovarian capsule ; *p.f*, primary follicle ; *r.f*, regressive follicle ; *th.f*, theca folliculi ; *u.g*, uterine gland ; *vg.e*, vaginal epithelium ; *z.p*, zona pellucida.

Fig. 1. Section of a virgin ovary—note the large number of primordial follicles.

Fig. 2. Section of the ovary of a specimen collected in February. Note the large number of developing follicles.

Fig. 3. The germinal epithelium of the same as fig. 2. It shows the occurrence of oocytes differentiated and sinking into the medullary part of the ovary. Hence they occur at various levels.

Fig. 4. A fully formed unilaminar follicle. Note the single layered theca folliculi.

Fig. 5. Bilaminar Graafian follicle with a complete investment of theca folliculi. Some of the follicular cells are undergoing division.

Fig. 6. Fully formed multilaminar Graafian follicle. Theca is made up of many layers of cells.

Fig. 7. Graafian follicle with the beginnings of the formation of the antrum as intercellular spaces.

Fig. 8. Section of a part of the ovary of a specimen collected in April. Note the follicles undergoing regression near the region of the hilus. In each case of the atretic follicle the process of degeneration starts with the ovum.

Fig. 9. A part of the ovary of a specimen with a free morula stage in the uterus. The corpus luteum occurs at one end and its centre is occupied by extravasated blood. The corpus luteum is in its initial stages of development.

Fig. 10. Section of the Fallopian tube of a specimen collected in September.

Fig. 11. Section of the Fallopian tube of a specimen collected in February. Note the enlarged epithelial folds.

Fig. 12. Section of the uterus of a specimen collected in September.

Fig. 13. Section of the uterus of a specimen collected in February. Note the large number of uterine glands opening into the uterine lumen. On one side the uterine epithelium is about to get ruptured due to over-hypertrophy.

Fig. 14. Section of the vagina of a specimen collected in September.

Fig. 15. Vagina of a specimen collected in February. Note the lumen filled with the desquammated cells. The vaginal epithelium is enormously hypertrophied and cornified.

FIG. 16. Section of a non-lactating mammary gland. Note the enlarged adipose cells, while the lactiferous alveoli are reduced to a negligible condition. Note the connective tissue fibres traversing the interglandular regions.

FIG. 17. Section of the mammary gland of a specimen at full-term pregnancy. Note the enlarged lactiferous alveoli and the pronounced collecting ducts.

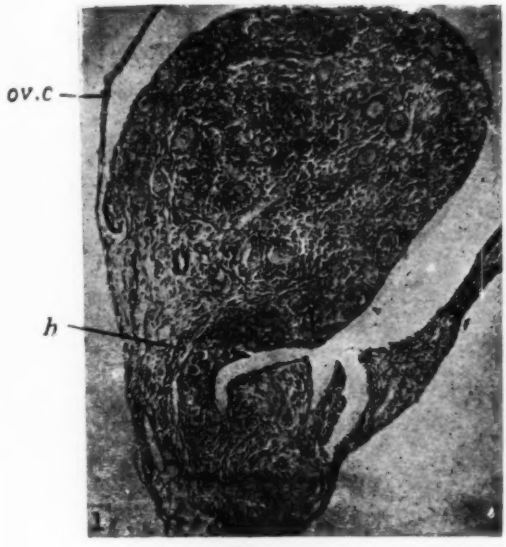
FIG. 18. Section of the ampulla of Henle of a specimen collected in September.

FIG. 19. Section of the ampulla of Henle of a specimen collected in February. See the large lumina of the glands filled with secretion.

FIG. 20. Section of the prostate of a specimen collected in September. The connecting tissue stroma occupies a large part of the gland. Secretory alveoli are reduced.

FIG. 21. Section of the prostate of a specimen collected in February. Note the enormously enlarged lumina of the glands filled with secretion.

FIG. 22. Section of the Cowper's gland at full activity from a specimen collected in February. The enlarged glandular alveoli are clearly discernible. Note the secretory droplets in the glands.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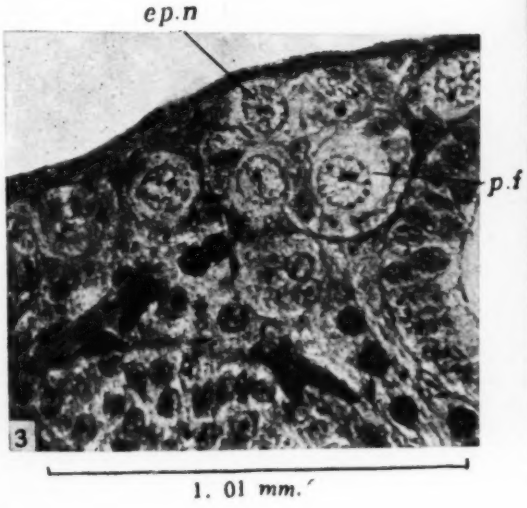
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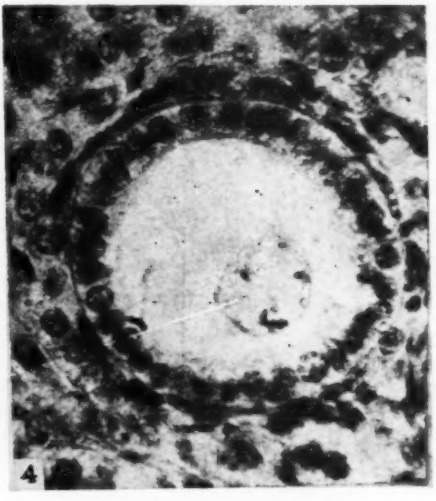
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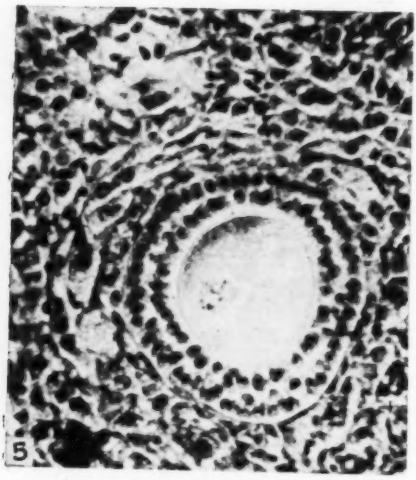

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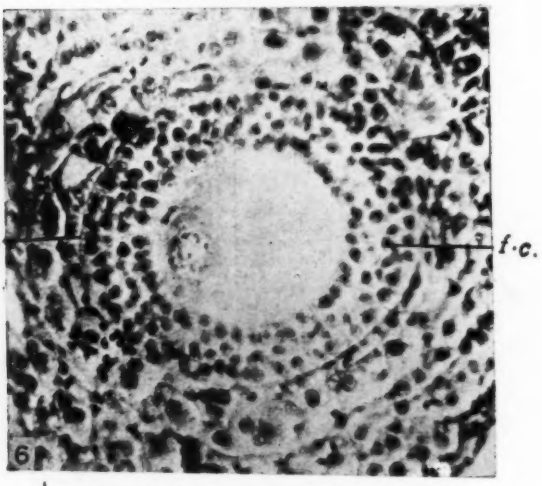

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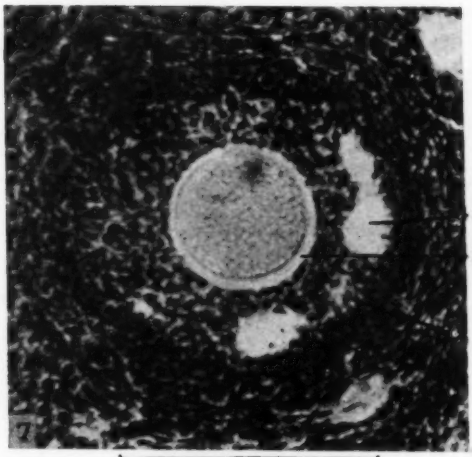
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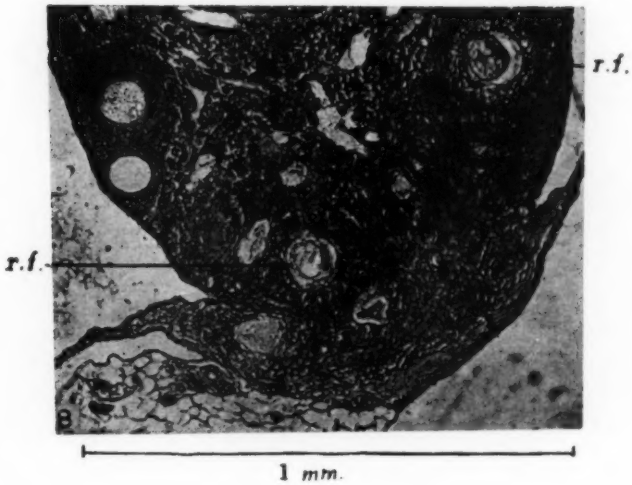
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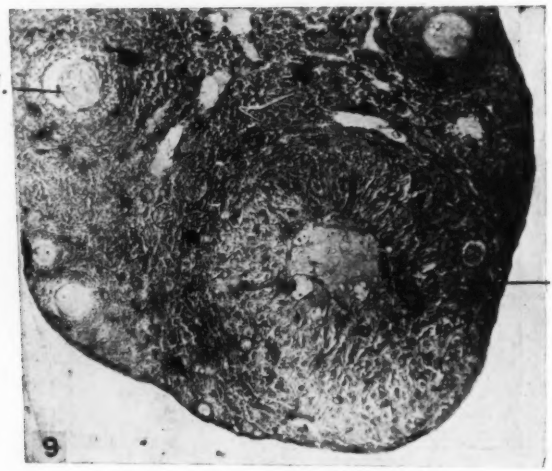

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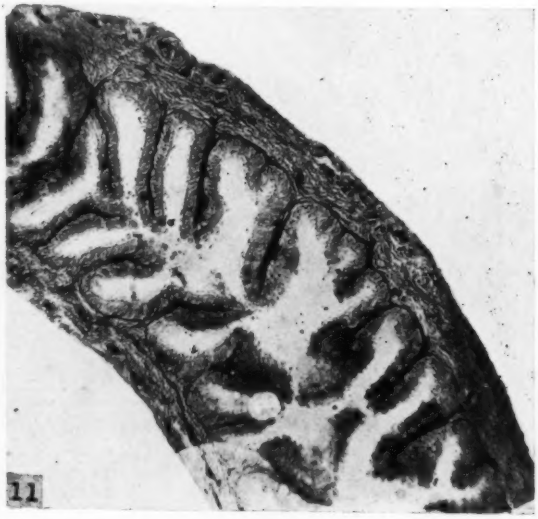

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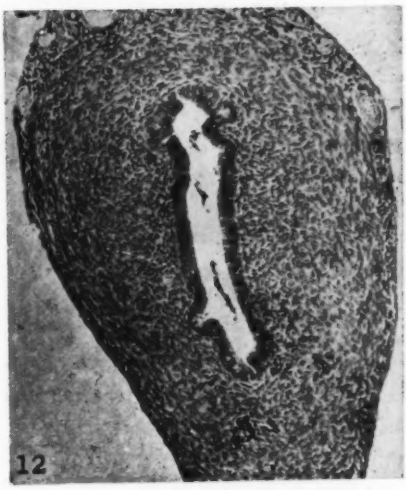
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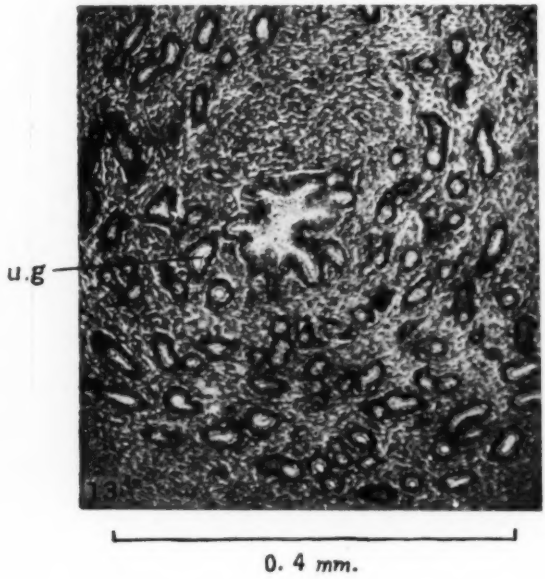
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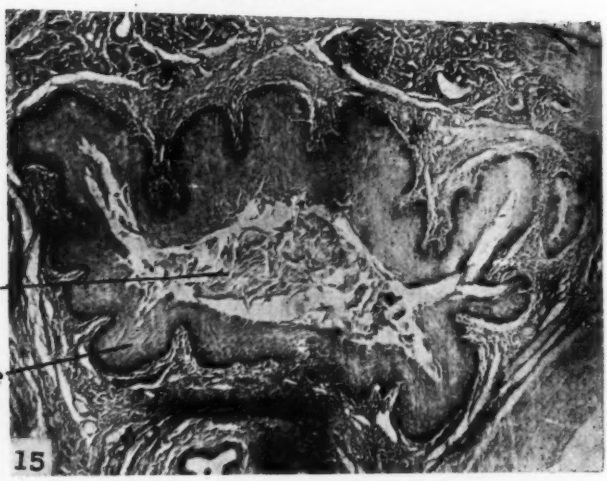

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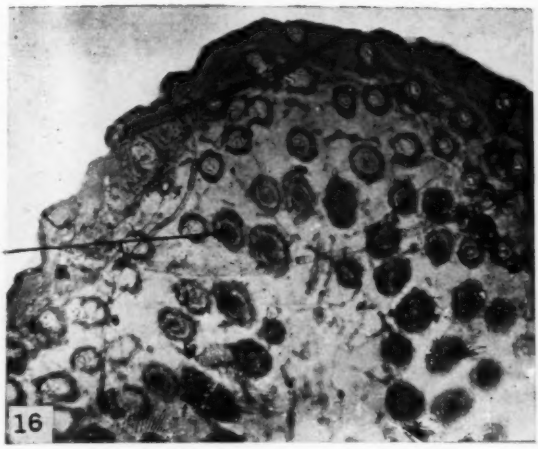

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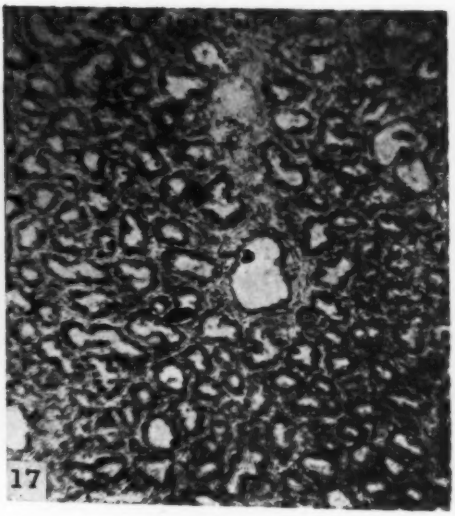

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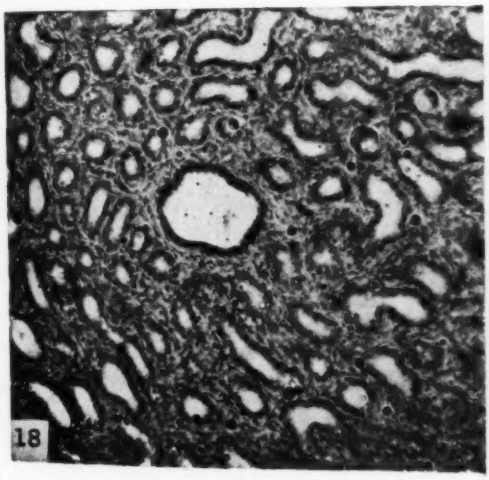

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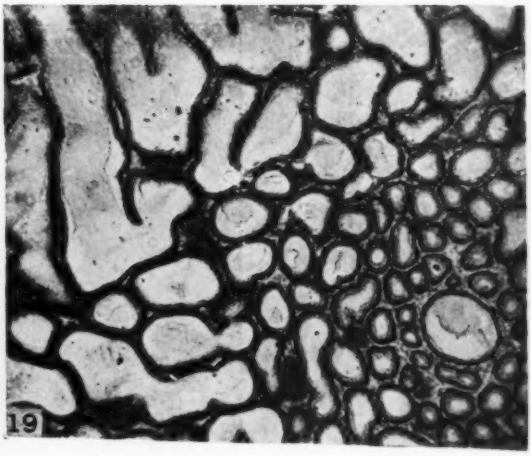

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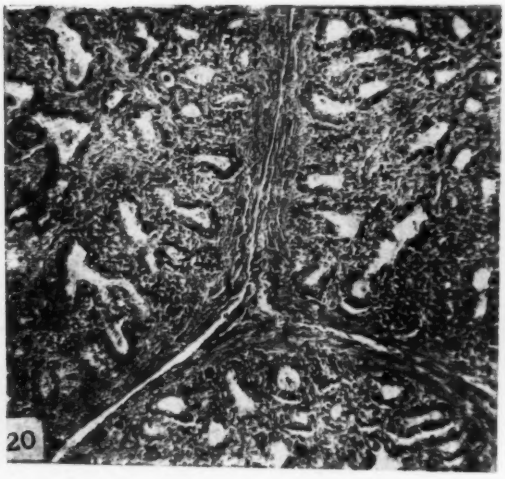

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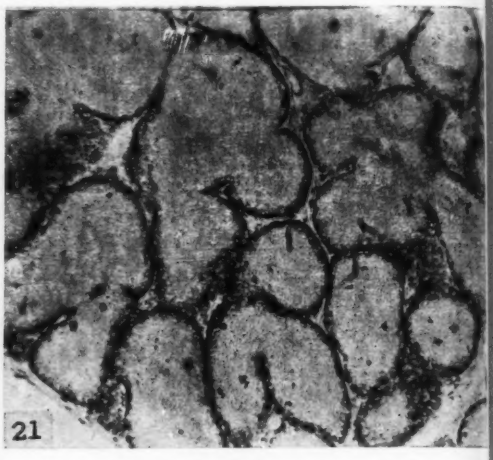

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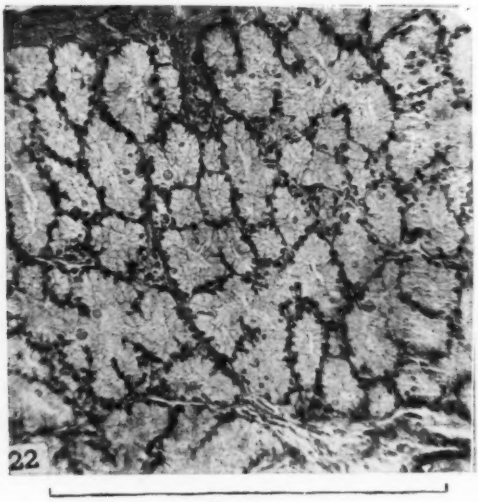

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THE ACTION OF IONS AND DRUGS ON THE EFFECTS OF ASPHYXIA ON THE MECHANICAL RESPONSE OF UNSTRIATED MUSCLE

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(From the Physiological Laboratory, Medical College, Agra)

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The susceptibility of unstriated muscle to asphyxia shows wide variations. Some muscles continue to respond in the absence of oxygen and glucose for several hours, while others become inexcitable in a few minutes. Sometimes every muscle experimented upon shows marked asphyxial augmentation of excitability, while at other times it is practically absent. In the present research an attempt has been made to find the cause of these variations in so far as ions and drugs may modify the effects of asphyxia on unstriated muscle.

Experimental

The muscles used were those from frog's stomach (Singh, 1939), dog's stomach (Singh, 1940) and fowl's gut (Singh, Singh and Muthana, 1946). The experimental procedure was as in previous papers (Singh and Singh, 1948 *a*). The response referred to is that produced by alternating current unless otherwise stated.

Results

Effect of hydrogen ions.—A glycolytic metabolic mechanism in acid solutions (experimental range, upto pH 4·4) has been found, as shown by the effect of glucose in relieving asphyxial arrest (Fig. 1) in causing marked increase in asphyxial exaltation of excitability (Fig. 2) and in improving the response

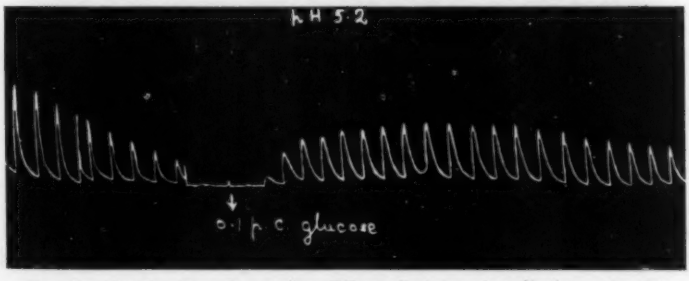

Fig. 1. Frog's stomach muscle. Effect of glucose in relieving asphyxial arrest at pH 5·2

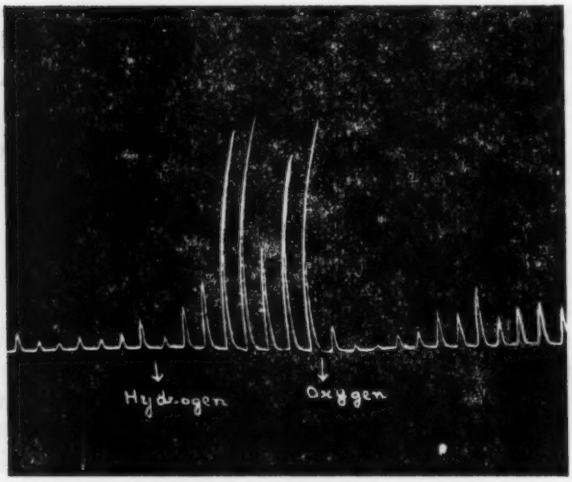

FIG. 2. Dog's stomach muscle. Effect of asphyxia at pH 6·5 in 0·1 *p.c.*, glucose

in the presence of oxygen. The effect is most noticeable upto pH 5 in frog's stomach, upto pH 6·5 in dog's stomach, and is not significant in fowl's gut. In dog's stomach the effect on asphyxial increase of excitability is very marked. On introduction of oxygen, there is depression of excitability before return to normal. This is most marked in dog's stomach. Thus the Pasteur effect is also present in acid solutions. The action of glucose is antagonised by iodo-acetic acid.

The glycolytic mechanism in acid solution is antagonistic to the non-glycolytic one, as shown by the inhibitory effect of glucose (Fig. 3).

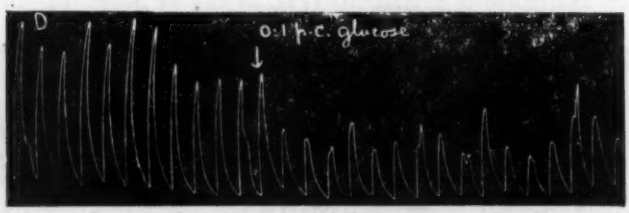

FIG. 3. Frog's stomach muscle. Effect of 0·1 per cent. glucose at pH 6·5

The glycolytic mechanism in acid solutions is not the same, but antagonistic to that in alkaline solutions. This is shown by the following observations:

(1) If the reaction is changed from alkaline to acid, then the action of glucose is suppressed; there is very little increase in asphyxial excitability.

On return to alkaline solution, the asphyxial increase in excitability occurs as usual (Fig. 4).

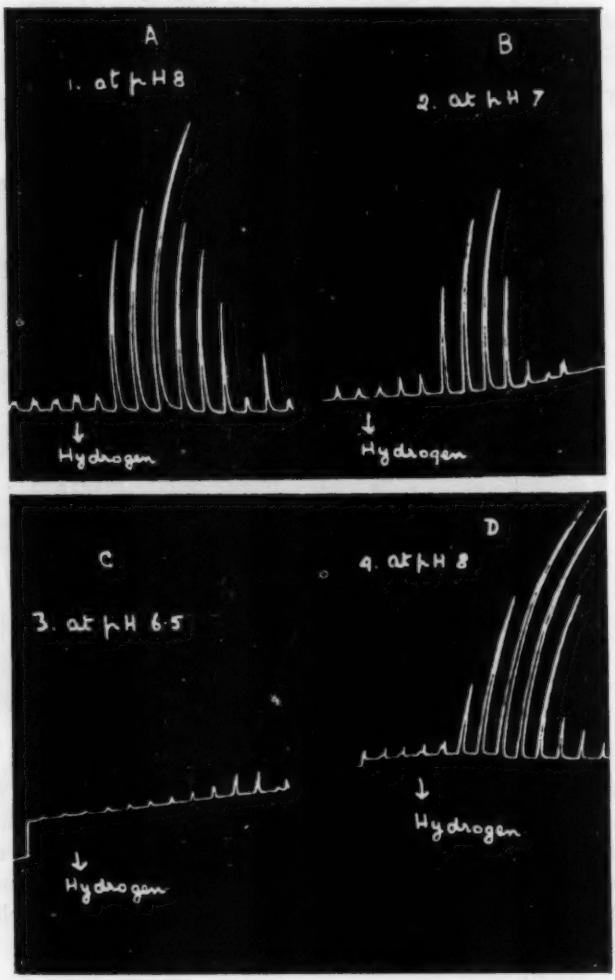

FIG. 4. Dog's stomach muscle. Asphyxiation in 0·1 per cent. glucose at pH 8, 7 & 6·5

(2) For the production of effects of glucose in acid solutions, the muscle has to be soaked for about an hour in the latter. This shows that the acid mechanism gradually takes over from the alkaline one. In some muscles the taking over is immediate.

The time lag in taking over by the acid from the alkaline mechanism probably accounts for changes in excitability caused by increase in hydrogen ion concentration of the saline. Thus by changing from alkaline to acid, the excitability is at first depressed and then increased or may increase immediately.

(3) If asphyxial arrest is produced either in alkaline or acid solutions, then change of reaction immediately restores the response (Fig. 5). The

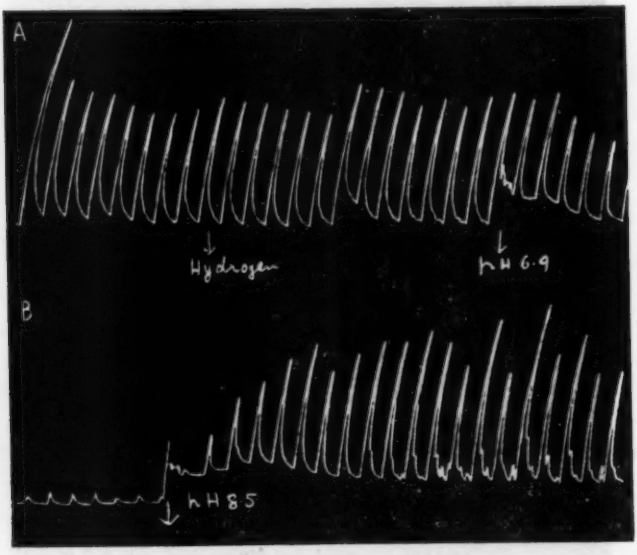

FIG. 5. Frog's stomach muscle. Effect of change of reaction on asphyxial arrest

response may be bigger than before indicating that the alternate mechanism had rested. In this way asphyxial arrest can be produced alternately in acid and alkaline solutions, and relieved by change of reaction. The experiment can be continued for over 4 hours, but the response gradually declines. These experiments show that the acid mechanism rests in alkaline solutions and *vice versa*.

The above is also shown by the action of iodoacetic acid. In iodoacetic acid no recovery takes place in the absence of oxygen, so that if the acid mechanism rests in alkaline solution, or the alkaline in acid solution, then iodoacetic should also prevent the recovery when the reaction is changed. Iodoacetic acid diminishes the recovery on change of reaction but does not abolish the response, so that other factors then rest are involved. This

additional recovery is probably due to the tone producing property of change of reaction.

The responses to potassium and acetylcholine in acid solutions are similar to those in alkaline solutions. The action of glucose is at first inhibitory and then stimulatory, though it may be stimulatory from the start. Asphyxial increase in excitability is also produced.

Adaptation is also diminished in acid solutions as a result of asphyxia (Fig. 6).

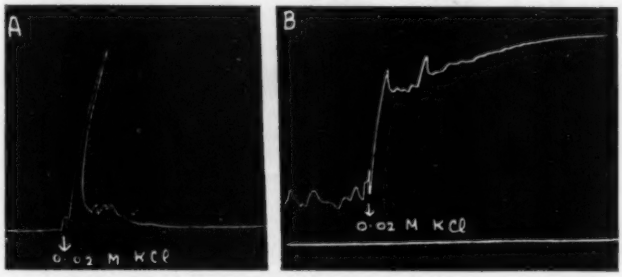

FIG. 6. Frog's stomach muscle.
A. Stimulation by 0·02 M KCl in presence of oxygen
B. Stimulation by 0·02 M KCl in asphyxiated muscle

The action of tone producing substances in relieving asphyxial arrest is more marked in acid solutions, and this effect is partially antagonised by glucose (Singh and Singh, 1948a, b, c.).

In acid solutions also, the anaerobic metabolic mechanism for tone and twitch appears to be different. Thus addition of glucose to relieve asphyxial arrest may result in increase of tone and decrease of twitch. The differences in aerobic mechanisms is shown by increase of tone on introduction of oxygen and decrease of twitch.

The difference between the anaerobic mechanisms of tone and twitch is also shown by the following experiments, both in alkaline and acid solutions.

(1) During asphyxial increase in excitability in the presence of glucose, there may be enormous increase in the twitch contraction of dog's stomach muscle (from 0-2 to 200 g.), without any increase in tone whatsoever, though a tonic contraction is otherwise easily produced such as by stimulation with acetylcholine or potassium. This shows that the glycolytic anaerobic mechanisms for tone and twitch are different.

(2) In the absence of glucose, or in the presence of iodoacetic acid, there may be similar increase in the twitch contraction during

asphyxia without increase in tone. This shows that the non-glycolytic anaerobic mechanisms for tone and twitch are different. If tone also increases, then it is alactic tone.

(3) If the muscle is asphyxiated tone may decrease, though the twitch remains unaffected or increases temporarily.

Both tone as well as twitch may be affected identically during asphyxia, showing that they may draw energy from a common source (Fig. 7).

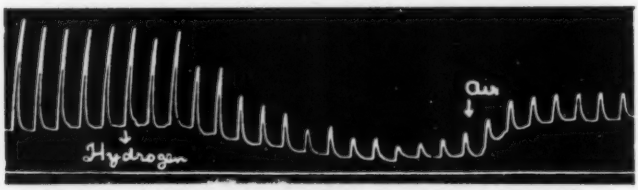

FIG. 7. Frog's stomach muscle poisoned with 1 in 1000 NaF. Effect of asphyxia on tone and twitch

Effect of potassium.—Excess of potassium not sufficient to cause inexcitability has two effects. Either the asphyxial arrest is postponed or hastened. The former is due to the usual effect of tone-producing substances, and is thus produced by other similar substances. The latter effect is due to opposite action of such substances (Singh and Singh, 1948 *b*.).

In the absence of potassium, asphyxial arrest occurs later than in its presence. This is probably due to diminution of excitability, so that the muscle is not exhausted soon. The effect of the power of contraction on exhaustion of muscle is shown in Fig. 8. The asphyxial increase in excitability is dimi-

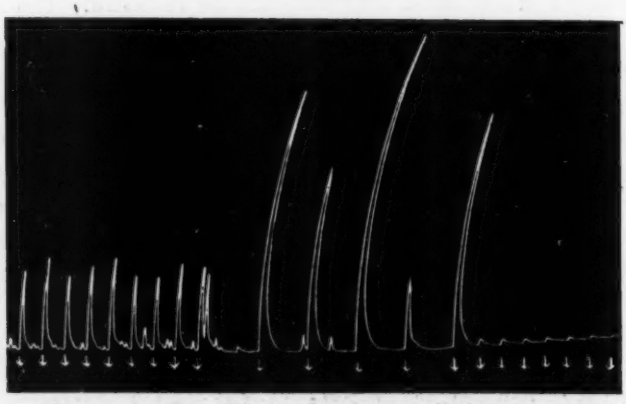

FIG. 8. Frog's stomach muscle. Effect of frequency of stimulation on asphyxiated muscle

nished in the absence of potassium; tone also shows lessened susceptibility to asphyxia (Fig. 9).

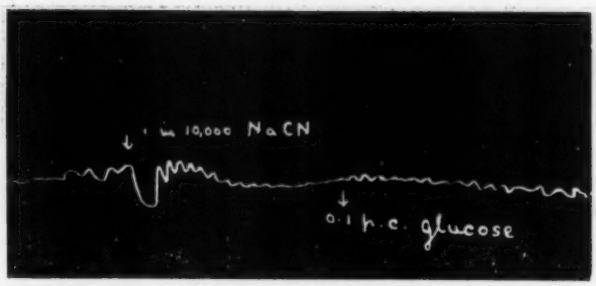

FIG. 9. Fowl's gut. Effect of 1 in 10,000 NaCN in absence of potassium

Effect of calcium.—In the absence of calcium, asphyxial arrest is postponed, probably due to diminished excitability. The asphyxial increase in excitability is decreased.

Effect of anions.—Br, I, NO_3, SCN, either postpone or hasten asphyxial arrest. The former effect is more marked in dog's stomach and the latter in frog's stomach.

Effect of vitamins.—Riboflavine slightly relieves asphyxial arrest and increases the response in the presence of oxygen (Fig. 10). This suggests that flavoproteins take part in respiration of unstriated muscle. Thiamine, nicotinic acid and ascorbic acid have no such effect.

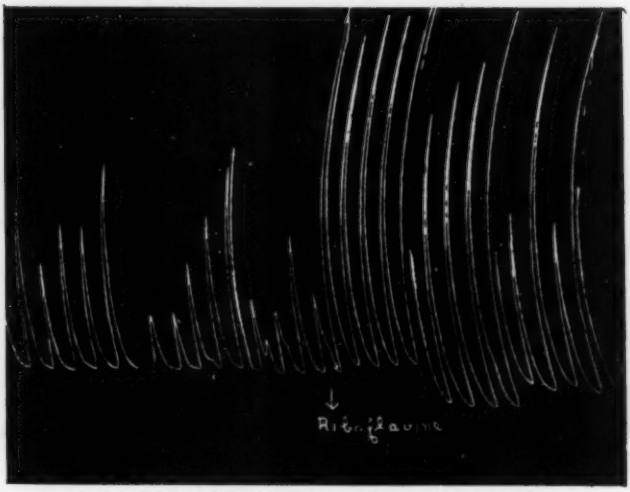

FIG. 10. Dog's stomach muscle. Effect of Riboflavine

Effect of osmotic pressure.—Sudden increase of osmotic pressure of the saline relieves asphyxial arrest (Fig. 11). This suggests that intracellular

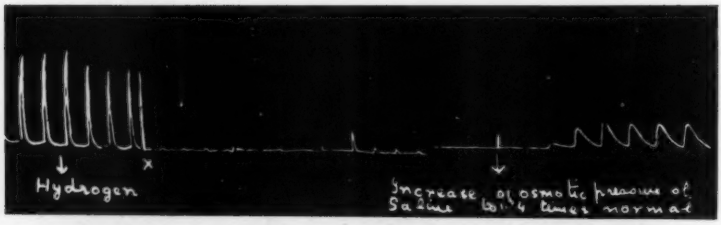

FIG. 11. Frog's stomach muscle. Effect of sudden increase of osmotic pressure of the saline to 1·4 times normal in relieving asphyxial arrest. Drum stopped at X for 15 minutes

potassium acts similarly to extracellular potassium. Its possible role in excitation has been discussed (Singh and Singh, 1948 *a*).

Effect of glucose.—Glucose, 0·1 per cent. may have an inhibitory effect also in the presence of oxygen. This shows that the non-glycolytic aerobic mechanism is antagonistic to the glycolytic one. On removal of glucose, the response increases temporarily and the asphyxial increase in excitability also produces great augmentation of the response.

Glucose at first has an inhibitory effect on lactic tone in the absence of oxygen (Fig. 12). This shows a non-glycolytic source of energy for lactic tone.

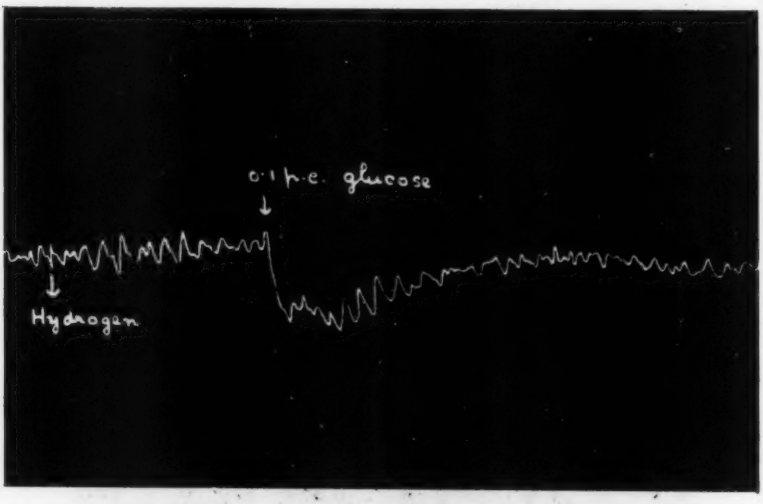

FIG. 12. Fowl's gut. Effect of asphyxia and glucose on tone

The nature of tone is determined by prolonged asphyxia. Lactic tone decreases whilst the alactic tone increases. Lactic tone may increase immediately on asphyxiation ; this is akin to the asphyxial increase in excitability to electric current, potassium and acetylcholine. This increase in lactic tone on asphyxiation should not be confused with increase of alactic tone ; glucose inhibits both. When the lactic tone begins to decrease, then glucose has a stimulatory effect, showing exhaustion of the non-glycolytic source. If glucose has a stimulatory effect from the start, it indicates non-functioning of the non-glycolytic source.

Effect of fatty acids, etc.—In the presence of oxygen, fatty acids such as sodium acetate, propionate and butyrate and sodium lactate improve the response both in alkaline and acid solutions. It therefore appears that facts are metabolised by unstriated muscle.

As there are several metabolic mechanisms in unstriated muscle, the question arises, as to the exhaustion of which of these produces asphyxial contraction. The asphyxial arrest of the response to electric current may not produce such a contraction. It is always produced in dog's stomach when exhaustion of a tonic contraction is produced. In frog's stomach, the same occurs, provided the muscle does not adapt to the substance producing tonic contraction. If the muscle adapts, then the asphyxial contraction does not occur, showing that adaptation is not due to exhaustion, but some change of excitability. Similarly it shows that fatigue may be due to two causes. One of these is due to exhaustion of energy stores as shown by the production of asphyxial contraction. The other is due to change in excitability similar to that in adaptation.

Inexcitability therefore may be due to two causes. One of these is due to a change in the excitatory mechanism and the other due to exhaustion of energy stores. These two states can be distinguished by increasing the strength of the stimulus. In the former state, responses occur, but not in the latter state, no matter how strong the stimulus. Thus in experiments on adaptation increase of strength of stimulus of alternating current from 8 to 16 volts, produces the original response. But in 'exhaustion inexcitability', no response occurs if the stimulus is similarly increased.

Discussion

Unstriated muscle which is a very complex tissue, also possesses very complex systems for metabolism. These may be classified according to the substance involved in metabolism, such as glycolytic and non-glycolytic, aerobic and anaerobic, or according to activity, such as for inhibition and contraction, for tone and twitch. Glucose is able to supply energy both

aerobically and anaerobically, and for inhibition, tone and twitch. The non-glycolytic system is probably the creatine phosphate system.

Summary

(1) There is a glycolytic system in unstriated muscle for acid solutions, as shown by the effect of glucose in improving the response both aerobically and anaerobically.

(2) The glycolytic system for acid solutions is antagonistic to that for alkaline solutions.

(3) Both the ærobic and anaerobic mechanisms for tone and twitch respectively are different both in acid as well as in alkaline solutions.

(4) Potassium postpones or hastens asphyxial arrest.

(5) Calcium also has similar action.

(6) Tone producing substances act similarly to potassium.

(7) Riboflavine improves the response aerobically as well as anaerobically. Thiamine, ascorbic acid and nicotinic acid have no such action.

(8) Sudden increase of osmotic pressure of the saline relieves asphyxial arrest.

(9) Glucose has inhibitory effect also in the presence of oxygen.

(10) Fatty acids, such as acetic, propionic and butyric also improve the response in the presence of oxygen.

(11) Inexcitability is of two kinds : one due to changes in the excitatory process, and other due to exhaustion of energy supplies.

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A METHOD FOR THE DETERMINATION OF CELLULOSE DESTROYING INDICES OF BACTERIA

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(Communicated by Dr. T. S. Subramanian, F.A.SC.)

Introduction

With the exception of the work of Reuszer,[1] no systematic study would appear to have been made, to determine the role of bacteria, in the deterioration of exposed cotton fabrics, although their wide distribution and potentiality for deterioration is well known. Recently, Reese[2] has shown, that under proper conditions of growth and nutrition, a large number of bacteria is capable of utilising cellulose. But no method has yet been recorded for the quantitative determination of loss in tensile strength (cellulose destroying index) of a cotton textile due to bacterial action. A suitable method is described in the present paper, which while simple in execution, yields at the same time reproducible results. The method can be also employed in the assessment of efficiency of rot-proofing agents against bacterial deterioration.

Method for the Determination of Cellulose Destroying Indices

A 250 c.c. Erlenmeyer's flask containing 50 c.c. of Omeliansky's medium[3] with a 2″ square piece of dosootie (basic fabric of Indian tentage weighing 6·4 oz., per sq. yd.) is sterilized. It is then inoculated with a loop of the individual test organisms isolated from different sources and incubated at room temperature for 7 days. One c.c. of this culture is added to a 500 c.c. Erlenmeyer's flask containing 150 c.c. of the same medium. A sterilised piece of dosootie $10\frac{1}{2}'' \times 2\frac{1}{2}''$ is introduced into the flask and incubated at room temerature for 15 days. A minimum of three replicates is used. After the incubation period, the samples along with the controls are removed, washed, dried, conditioned at 67% R.H. for 48 hours and the breaking strength ($2'' \times 7''$ between grips) determined in a "Goodbrand" machine.

Table I shows the cellulose destroying indices of different isolates of bacteria and illustrates the nature of results obtained.

TABLE I

Cellulose destroying indices of different isolates of bacteria

Organism No.	Breaking strength in lbs. of replicates 2" × 7" between grips after normal conditioning at 67% R.H.	Av. B.S. in lbs.	% Loss over control
(Control) ..	140, 140, 125, 125, 145, 125	133·0	..
2 ..	0, 35, 10	15·0	88·7
3 ..	55, 45, 40	46·6	65·0
5 ..	60, 65, 70	65·0	51·2
6 ..	20, 30, 30	26·0	80·0
8 ..	130, 130, 130	130·0	2·4
9 ..	0, 0, 45	15·0	88·7
10 ..	25, 20, 35	26·0	80·0
11 ..	10, 10, 20	13·0	90·0
12 ..	10, 10, 10	10·0	92·5

An examination of the results would indicate that although there are variations in the replicate figures for tensile strength, such variations are not wide and in any case are of the same order for both the control and the assays. The results are a measure of the standardisation reached in the method.

In order to test the reproducibility of the results as obtained by this method, experiments were carried out with some of the same organisms at different intervals and the results are given in Table II.

TABLE II

Reproducibility of cellulose destroying indices of bacterial isolates

Organism No.	Results from Table I (Experiment carried out in Oct. 1947). % Loss in B.S.	Results from experiment carried out in Jan. 1948. % Loss in B.S.	Results from experiment carried out in March 1948. % Loss in B.S.
2 ..	88·7	80·9	88·9
9 ..	88·7	94·2	85·4
11 ..	90·0	88·5	88·0
12 ..	92·5	94·2	94·4

Table II shows that the results of the method are reproducible.

APPLICATION OF THE TECHNIQUE FOR ASSESSING THE EFFICIENCY OF ROTPROOFING AGENTS AGAINST BACTERIAL DETERIORATION

Soil burial has been considered to be a very severe test for evaluating the efficiency of rotproofing agents and it is one of the standard test procedure

adopted in America.[4] Rapid deterioration of textiles in contact with soil is mainly due to the activity of cellulose destroying bacteria. When a fabric is treated with an effective rotproofing agent like copper naphthenate, it shows resistance to deterioration. To test the applicability of the present method in determining the efficiency of rotproofing treatment in general against bacterial deterioration, the following experiment was carried out.

Grey canvas cotton light (18 ± 1 oz. per sq. yd.) was treated with a solution of copper naphthenate in white spirit so as to leave 0·3% copper on the weight of the fabric. Replicates of three strips along with untreated controls were subjected to the action of isolates of four test organisms according to the method described above. The results are given in Table III.

TABLE III

Evaluation of rotproofing against bacterial deterioration

Organism No.	Grey canvas inoculated with bacteria					
	Untreated			Treated with copper naphthenate		
	B.S. 2" × 7" bet. grips in lbs.	Av. B.S.	% Loss	B.S. 2" × 7" bet. grips in lbs.	Av. B. S.	% Loss
Control ..	345, 550, 550	481·0	..	355, 325, 395	358·3	..
2 ..	85, 35, 40	53·0	88·9	375, 385, 385	382·0	−6·5
9 ..	45, 85, 80	70·0	85·4	415, 360, 380	385 0	−7·4
11 ..	90, 25, 140	85·0	82·3	380, 395, 390	388·0	−8·3
12 ..	80, 0, 0	26·0	94·5	395, 395, 360	383·0	−6·9

From Table III it may be seen that all the untreated pieces have shown a high degree of deterioration while none of the treated pieces show any loss in tensile strength, indicating the efficiency of the rotproofing treatment towards bacteria. Since the rotproofing treatment is a standard one, the results reflect on the suitability of the method for such assessment.

SUMMARY

A simple method has been described for determining the cellulose destroying indices of bacteria. The method may be employed in assessing the efficiency of rotproofing agents against bacterial deterioration.

ACKNOWLEDGEMENT

The authors desire to record their grateful thanks to Dr. T. S. Subramanian for his interest in the work and to the Deputy Chief of the General

Staff (W. and E.) and the Director of Technical Development, Army Headquarters (India), for kind permission to publish the paper.

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STUDIES IN CONTACT TOXICITY

Part I. Resistance of Successive Generations of *Culex fatigans* Wied. to Contact Insecticides

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[*Entomology Group, Technical Development Establishment, Laboratory (Stores), Kanpur*]

Received September 14, 1948

(Communicated by Dr. T. S. Subramanian, F.A.Sc.)

Introduction

In the course of investigations[1] on the evaluation of residual effect (persistence) of films of dichloro diphenyl trichloroethane (DDT) and benzene hexachloride (Gammexane) against *Culex fatigans* Wied., two new facts, not germane to the main evaluation work, have been brought to light. A careful study has shown that these relate to (1) a type of progressive resistance (to insecticide) shown by the test insects drawn from successive generations of parent culture and (2) reactivation of surfaces treated with DDT as a result of storage of such surfaces in a Burma teak box. Available evidence has indicated that these two effects are independent of each other, and in view of their importance in laboratory study of the toxicology of insecticides, they are reported in detail in this series of papers. In the present paper are described the technique of evaluation of contact toxicity using standard breed of *Culex fatigans* as also the experiments leading to the discovery of increased resistance (to contact insecticides) shown by successive generations of the standard test insect.

Material and Technique

1. *Test Insects* (Culex fatigans *Wied.*)

The mosquitoes were bred in a room conditioned at $85 \pm 1°$ F. and $72 \pm 3\%$ R.H. To obviate any variation in resistance to insecticides due to genetic factors, the colony was initially raised on 17–9–45 from one egg raft obtained from a single gravid female brought from the field. Blood meal for the ovipositing females was provided by a chicken kept inside the breeding cage. Egg rafts were collected from the breeding cages daily and transferred to cylindrical glass dishes 6″ diameter, each containing 300 c.c.

of tap water. The larvæ hatched out after 30 hours, and the population of the larvæ was adjusted to about 300–400 in each dish, containing in addition to water 0·15 gm. of powdered brewers yeast. After the second larval stage the concentration of the yeast in each dish was increased to 0·2 gm. Additions of yeast to the larval dishes were made daily. A light of uniform intensity was maintained over the breeding dishes. The pupation commenced on or about the eighth day of hatching of the larvæ. The pupæ were collected every two hours daily and transferred to a cylindrical dish (3″ diameter and 2″ deep) containing water to half its depth. Each two hourly collections were placed in wire-frame cages, 9″ × 9″ × 9″ covered with cloth on five sides and mosquito netting on the sixth side. A raisin or two, soaked and swollen in water, was placed inside the cage and served as food. The adults emerged 36 hours after the introduction of pupæ and were matured for 36 ± 2 hours longer when the normally healthy females were picked out and used as test insects.

2. *Testing Technique*

Test Chamber.—The test insects were exposed in a chamber composed of six glass plates, 3¼″ × 4¼″ × 1/16″, treated under identical conditions. The plates were built up in grooved blocks on a wooden base into a box, and the side plates were held together by a rubber band. The choice of glass as the surface for the deposition of the insecticide was governed by the fact that this material was convenient to handle, was uniform and facilitated microscopic examination of the insecticide, when necessary. The plates were treated uniformly on one side with the insecticide and the whole of the internal surface of the test chamber was formed by the treated surfaces. Uniformity of the deposit on the glass was achieved by spraying the requisite quantity of the insecticide, in solution or emulsion state, through a Potter's tower head[2] into a closed rectangular chamber, 2½′ × 2½′ × 4′, and allowing the mist to settle on the glass plates disposed horizontally on the bottom of the chamber. Chemical assay of the deposit on the plates showed that there was no significant variation between the plates.

Preparation of the test insects for exposure.—Preliminary investigations showed that when winged adult mosquitoes were let into a chamber as described above, the contact of the insects with the insecticide (DDT) stimulated their flight, thereby introducing a variable factor in relation to the total contact period of the different insects in one batch. Further, as a result of flight, the insects knocked against the plates and possibly picked up greater quantities of insecticide than they would do otherwise. The

possibility of mechanical injury to the insects could not also be excluded. It, therefore, appeared that it would be more convenient to use for test purposes insects whose wings had been clipped.

For this purpose the insects were lightly anæsthetised. They were initially collected in a sucking tube, the opening of the tube placed in the narrow mouth of a bottle containing chloroform and the vapour sucked in for the fraction of a second. The insects were held in the tube for about 40 seconds and then tipped out on to a sheet of blotting paper. About two-thirds of each wing was then clipped off by means of scissor forceps. Complete recovery from the effects of anæsthesia took place in about thirty minutes as the following table would show. A similar period was allowed to elapse before the insects were used for test. A total of twenty insects was used in each test.

TABLE I

Effect of chloroform on mortality of mosquitoes exposed to glass plates treated with DDT

(Concentration of deposit 35 mgm./sq. ft.)

Interval between the administration of chloroform and exposure in minutes	Mortality percentage (Contact Period 15 minutes)
30	50
60	55
120	50
180	55

It was realised that the administration of anæsthetic and clipping of wings may influence the susceptibility of the insect to the insecticide. The exposure of winged mosquitoes in the test chamber was likely to lead to inaccuracies as pointed out earlier, and obviously this method was not suitable for investigating this point. Recourse was made to a technique described by Major Iles (private communication) in which the contact of the winged insect with the insecticidal surface forming the base of a glass cylinder was ensured by rotating the walls and top of the cylinder and maintaining the base stationary. Different contact periods were investigated and concurrent tests were carried out on insects whose wings had been clipped. Controls were run on untreated surfaces. Table II summarises the results obtained.

Although contact periods greater than 60 seconds have not been investigated, the above results are indicative of the fact that the joint effect of

TABLE II

Difference in susceptibility of winged and wingless mosquitoes to DDT applied from solution in crude kerosene

(Concentration 34 mgm./sq. ft.)

Age of insecticidal film (Days)	Contact period in seconds	Percentage mortality		
		Winged	Wingless	Control
1	10	100	100	0
3	10	100	90	0
4	10	50	60	0
11	20	30	30	0
13	30	10	10	0
16	60	0	0	0

anæsthesia and clipping of wings in altering the susceptibility of the insect to the insecticide is negligible.

Age of test Insects.—It has been stated in the earlier section on breeding of the test insects that normal healthy adults (females) 36 ± 2 hours old were used for tests. The choice of this particular age of adults was the result of a preliminary study to investigate the variation in resistance in the different age groups of adults. Table III below summarises the results obtained.

TABLE III

Susceptibility of mosquitoes of various age groups to filter papers impregnated with DDT

Age group of adults (in hours)	Concentration (in mgm./sq. ft.)	Contact period (in minutes)	Percentage mortality*
11–20	1975	60	25
26–35	2005	60	25
48–66	1920	60	35
80–86	1860	60	5

* Mortality in control in all cases was zero.

An examination of the results would show that the resistance of the insect is extremely high when it is about 80 hours old. It was considered that insects 36 ± 2 hours old should prove suitable for test purposes.

Exposure in the chamber.—The insects were released into the chamber and after a definite period of exposure were transferred by means of a sucking tube to a filter-paper resting on a Petridish and then covered over.

A lamp chimney is suitable for this purpose. Food was provided and consisted of a raisin which had been soaked in water previously. The mortality of the insects twenty-four hours after the exposure was recorded.

Controls were run concurrently with the experimental assays. This consisted of exposure of insects (with wings clipped) to unsprayed plates for the same contact period as in the assay. Here again the mortality after 24 hours was noted.

The tests were carried out in the same room in which the insects were bred.

3. *Insecticides*

A 5% DDT (dichloro-diphenyl trichloroethane) emulsion in water prepared by suitable dilution of the following miscible oil concentrate was used for spraying plates:—

DDT	..	25	by weight (pp' isomer content 65%)
Toluene	..	32·4	,,
Turpentine	..	32·4	,,
Alcohol (Absolute)	..	5·3	,,
Soap Powder	..	3·4	,,
Distilled Water	..	1·5	,,

At one stage of the investigation a 5% emulsion of benzene hexachloride (Gammexane) derived from a concentrate similar to above was also used.

Results

1. *The Effect*

Table IV below records the percentage mortality of mosquitoes exposed to glass plates treated with D.D.T. derived from a single concentrate prepared on 19–12–45.

Table IV

Date of treatment by mist deposition technique	Date of exposure of mosquitoes	DDT concentrate mg./sq. ft.	Contact period in minutes	Percentage mortality*
19–12–45	20–12–45	35	20	100
			30	100
3–1–46	4–1–46	37	20	60
5–4–46	6–4–46	37	20	15
			30	30
20–5–46	21–5–46	50	20	0
			30	15
22–5–46	23–5–46	38	20	0
			30	0
23–5–46†	24–5–46	51	30	0

* Mortaliy in control in all cases was zero.

† The DDT content in the concentrate had not altered during the storage period 19–12–45 to 23–5–46.

Table IV shows that although on 20–12–45 a hundred per cent. mortality was recorded, after six months there was no kill at all for the same contact period, *viz.*, 30 minutes. Further, the fall in mortality rate was gradual. Confirmation of this was sought and Table V below summarises the results obtained with a fresh emulsion concentrate prepared on 24–5–46.

TABLE V

Date of treatment with DDT by mist deposition technique	Date of exposure of mosquitoes	DDT concentration mgm./sq. ft.	Contact period (Minutes)	Percentage mortality*
24–5–46	25–5–46	40	30	65
28–5–46	29–5–46	36	30	65
30–5–46	31–5–46	45	30	50
10–6–46	31–5–46	55	30	25
21–6–46	22–6–46	35	30	20
1–7–46	2–7–46	59	30	20
12–7–46	13–7–46	41	30	10
1–8–46	7–8–46	44	30	0

* Mortality in control in all cases was zero.

Whilst a percentage mortality of 65 for 30 minutes contact on the first exposure (25–5–46) was unexpected in view of the results in Table IV it is nevertheless clear that the same type of increasing resistance as illustrated in Table IV is also manifest in the results of Table V. It would appear that possibly more than one factor is responsible for these results.

2. *Influence of the Purity of DDT on the Effect*

In attempting to trace these factors it was necessary to take note of the possibility of any deterioration in the quality of DDT during storage as emulsion concentrate. It was considered of interest, therefore, to investigate the influence of purity of DDT on mortality rate. Table VI records the observations on this aspect. The pure pp′ isomer of DDT was employed. The date of preparation of the miscible oil concentrate was 1–7–46.

TABLE VI

Date of treatment by mist deposition technique	Date of exposure to mosquitoes	DDT concentrate mgm./sq. ft.	Contact period (Minutes)	Percentage mortality*
1–7–46	2–7–46	39	20	0
			30	0
25–7–46	26–7–46	38	30	0
25–7–46	26–7–46	35	30	0

* Mortality in control in all cases was zero.

Table VI shows the great resistance offered by the insects to films containing hundred per cent. pp' DDT. Further it makes it clear that the state of purity of DDT, *per se* is not a factor connected with the increased resistance.

3. *Behaviour towards Gammexane*

In order to confirm that the effect was independent of the nature of insecticide, experiments were carried out using benzene hexachloride (Gammexane). The emulsion containing 'Gammexane' was prepared from fresh miscible oil concentrate on each occasion the plates were sprayed. The results are described in Table VII.

TABLE VII

Date of treatment by mist deposition technique	Date of exposure to mosquitoes	Gammexane concentration mgm./sq. ft.	Contact period (Minutes)	Percentage mortality*
3-1-46	4-1-46	34	5	100
18-9-46	19-9-46	31	5	40

* Mortality in control in both the cases was zero.

Table VII indicates that the effect is valid for 'Gammexane' also.

DISCUSSION

For any accurate and valid toxicological assay of contact insecticides it is essential that (1) the test insects are standard and (2) the deposit of insecticide against which the insects are tested is uniform. Both these requirements have been satisfactorily met in the experiments recorded in the present paper. A careful analysis of the results obtained however, point to the fact that successive generations of a standard culture of *Culex fatigans* exhibit increasing resistance to a contact insecticide like DDT. At the end of about a year after the start of culture, the resistance is very pronounced. A concentration of 35 mgm./sq. ft. of DDT leads to hundred per cent. mortality of the test insects on 20–12–45, when the contact period is 30 minutes, whereas a concentration of 44 mg./sq. ft. leads to zero per cent. mortality on 7–8–46 for a similar contact period. The exact cause of this increased resistance is not understood but it would appear that the resistance is not peculiar to DDT alone. It is valid for benzene hexachloride (Gammexane) also. It may be that this effect is part of a rhythmic variation in the resistance of *Culex fatigans*. It is hoped to investigate this aspect in detail.

Summary

A technique for the breeding of standard *Culex fatigans* for toxicological studies as well as a method for the evaluation of toxicity are described. Experiments have shown that successive generations of the test insects show progressive resistance to a contact insecticide. The progressive resistance to DDT over a ten-month period is recorded.

Acknowledgement

The authors' thanks are due to Dr. T. S. Subramanian for his interest in the work and to the Deputy Chief of General Staff (Weapons and Equipment) and the Director of Technical Development, Army Headquarters, New Delhi, for kind permission to publish the paper.

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A PETROGRAPHIC STUDY OF THE ALKALINE ROCKS AT SIVAMALAI*

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Abstract.—Sir Thomas Holland proposed the term *Sivamalai Series* to include a group of alkaline rocks which are prevalent in the neighbourhood of Sivamalai in the Coimbatore District. Since the publication of Holland's Memoir little attention has been paid to them. As a result of detailed petrographic study of the rock types of this area a geological map has been prepared by the present writer (see map appended). Three types of rocks and a rose coloured variety of nepheline have been analysed.

The rock suite is characterised by soda-rich feldspars and pyroxenes, the feldspars being mostly varieties of crypto and microperthite, of the exsolution and replacement types. The plagioclase feldspars in these rocks have 13–18% 'An' content and invariably exhibit the Albite-Ala twin law. The rose-coloured nepheline was found to be particularly rich in soda and having low indices of refraction. The pyroxene in the pyroxene syenites is pleochroic ægirine-augite, occurring intimately associated with olivine and hypersthene. Three varieties of amphibole were noticed in these rocks, one of which is similar to 'Girnarite', a species named by Mathur.

Eight distinct types of rocks have been recognised on the basis of detailed petrographic examination, these being (1) coarse nepheline syenite, (2) aplite-syenite, (3) biotite-nepheline-syenite, (4) hornblende-nepheline-syenite, (5) hornblende-nepheline-syenodiorite, (6) even-grained granulitic nepheline-syenite, (7) ægerine-augite-syenite, and (8) microperthitite.

Rock types 3, 5 and 7 have been analysed and these together with an analysis given by Holland have been plotted on the 'QLM' and 'πk' triangular diagrams after Niggli. These diagrams bring out the undersaturated nature of these rocks and their affinity to the Atlantic Suite. Field evidence suggests a syntectic origin for these rocks, but for a fuller understanding of the genesis of the suite it will be necessary to undertake more extensive chemical studies.

* Substance of a thesis submitted for the M.Sc. degree examination, Benares University, 1942.

General

Introduction.—Middlemiss (1896) described some pale grey gneisses at Sivamalai (58 E/12—11° 2′ : 77° 32′ 30″) in the Coimbatore District, Madras, composed wholly of granular plagioclase and microcline, with biotite or hornblende, iron oxides and zircon as accessories. A few years later, Sir Thomas Holland (1904) discovered the presence of nepheline and graphite in these rocks and, as they comprised several alkaline types, gave the suite the name of "Sivamalai Series". Since the publication of Holland's classical Memoir on these rocks, little attention has been paid to them, though the genesis of alkaline rocks in general has been one of the most controversial petrological problems. At the suggestion of Dr. K. P. Rode, the study of these rocks was taken up by the author and carried out in the field and the Geology Laboratory of the Benares Hindu University in 1941–42. Owing to war-time conditions a comprehensive petro-chemical investigation was not possible, but the petrography of the rocks received careful study, the results of which are presented here.

A preliminary field study of the area was made in October 1940 which was followed by intensive fieldwork in June 1941. The alkaline rocks were mapped on the scale of 4″ = 1 mile in a specially enlarged map, and a detailed collection of rock types made. About 120 rock specimens were collected from the main hill at Sivamalai at more or less regular intervals. Detailed petrographic study of these specimens has helped in evolving a generalised map of the hill indicating the distribution of the more important types.

The writer takes this opportunity of expressing his thanks to Dr. Raj Nath, Head of the Department of Geology in the Benares University, for the facilities given to him to carry out these investigations, to Dr. Rode, Dr. Chiplonkar and Mr. C. Rajagopalan, in the same institution, for several useful suggestions given to him during the study, and to Mr. S. Narayanaswami who accompanied him during field studies of the area.

Geologic setting.—Sivamalai (1,363 ft.) is a hill three miles NW of Kangayam (11° 0′ : 77° 34′) in the Dharapuram taluk of the Coimbatore District, standing out prominently amongst a dozen hillocks and knolls which extend in a west-north-westerly direction towards Kandampalaiyam (11° 2′ 30″ : 77° 28′). The country rock is a granitic gneiss with inclusions of biotite schist, and the nepheline-syenite occurs in the gneiss as lenticular bodies. These syenites are well foliated near the intrusive contacts, being parallel to the strike of foliation of the gneisses which is WNW-ESE. The darker minerals of the prevalent variety which is a biotite-nepheline-syenite have a tendency to linear arrangement roughly parallel to the foliation direction.

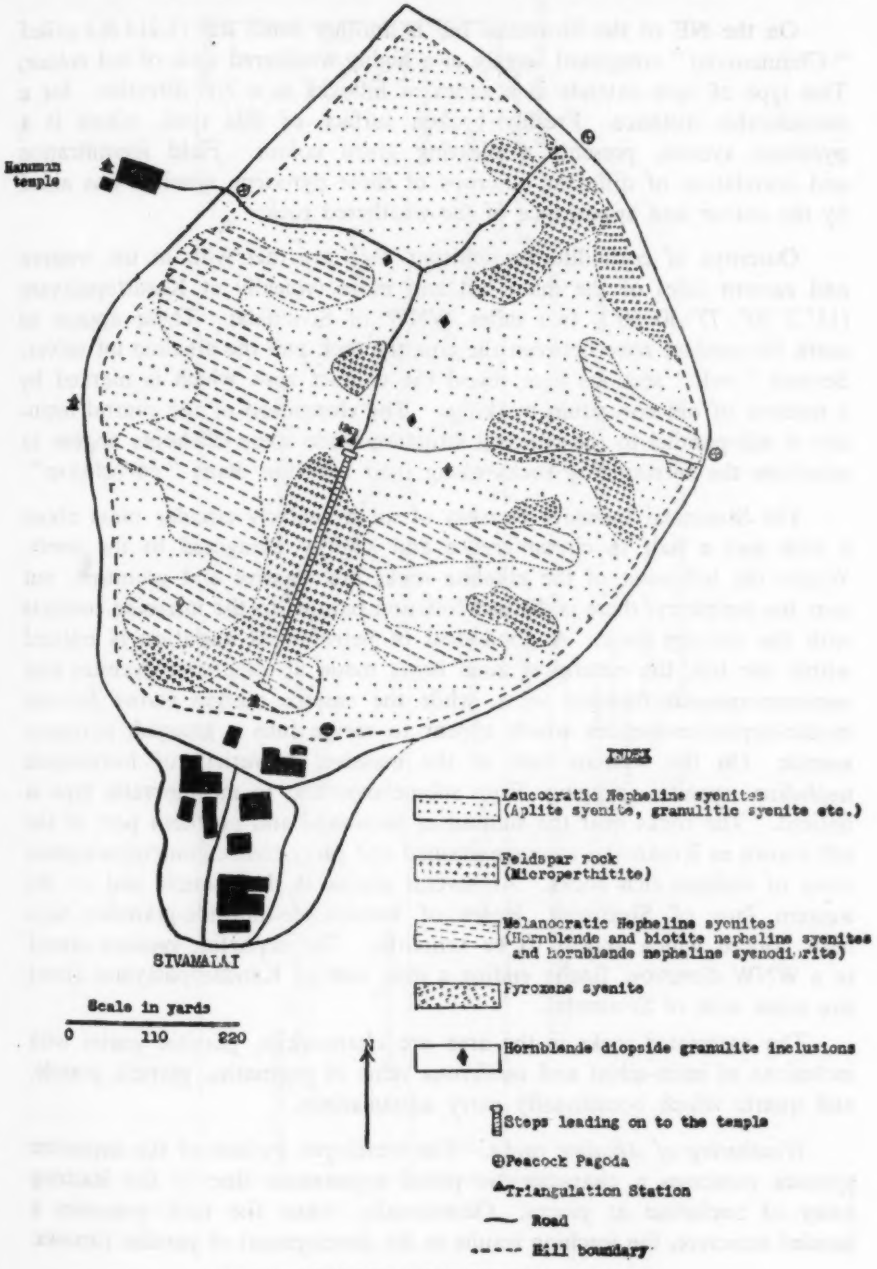

FIG. 1. Geological Sketch Map of Sivamalai Hill

On the NE of the Sivamalai hill is another small hill (1,214 ft.) called " Chinnamalai " composed largely of a highly weathered rock of red colour. This type of rock extends in a series of hillocks in a NE direction for a considerable distance. Freshly broken surface of this rock, which is a pyroxene syenite, presents a pleasing green colour. Field identification and correlation of different outcrops of these pyroxene syenites was aided by the colour and appearance of the weathered rock.

Outcrops of corundiferous feldspar rock are met with in the western and eastern sides of the main hill and more typically at Karattupalayam (11° 2′ 30″ : 77° 30′ 30″), two miles WNW of Sivamalai. These appear to mark the contact zone between the country rock and the alkaline intrusives. Several " well " sections here reveal the contact zone which is marked by a number of old corundum workings. The elongation of the central intrusive is sub-parallel to the regional structure while some offshoots appear to penetrate the surrounding rocks along their foliation plane " en echelon ".

The Sivamalai intrusive consists of an irregularly circular mass about a mile and a half in circumference and slightly elongated to the north. Within the hill most of the alkaline rocks are massive and granitoid, but near the periphery, there is distinct foliation parallel to the intrusive contacts with the country rock. A good deal of petrological variation is noticed within the hill, the outermost zone being made up of coarse syenites and contemporaneous feldspar veins, while the eastern margin shows foliated biotite-nepheline-syenites which appear to merge into a greenish pyroxene syenite. On the western face of the massive, a variety of hornblende nepheline syenites ranging from submelanocratic to melanocratic type is noticed. The rocks near the summit of Sivamalai and northern part of the hill known as Kosamalai are even grained and carry coarse contemporaneous veins of feldspar-rich rocks. At several places at the summit and on the western face of Sivamalai, lenses of hornblende-diopside-granulite have been noticed which appear to be xenoliths. The nepheline syenites extend in a WNW direction, finally ending a little east of Kandampalayiam about five miles west of Sivamalai.

The associated rocks in the area are charnockite, granitic gneiss with inclusions of mica-schist and numerous veins of pegmatite, graphic granite, and quartz which occasionally carry aquamarines.

Weathering of Alkaline rocks.—The weathered surface of the nepheline syenites possesses a characteristic pitted appearance due to the leaching away of nepheline at places. Occasionally, when the rock possesses a banded structure, the leaching results in the development of parallel furrows.

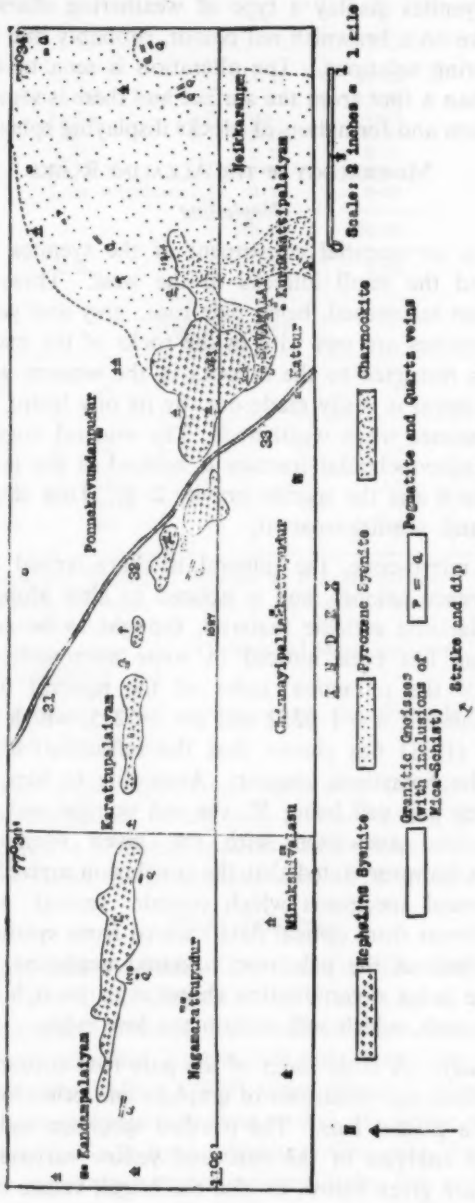

FIG. 2. Geological Map of Sivamalai Area

The pyroxene syenites display a type of weathering characteristic of charnockites and take on a brownish red colour, probably due to heavy staining by the iron-bearing solutions. The alteration is seen to extend in certain cases to more than a foot from the surface and there is sometimes a development of exfoliation and formation of blocks displaying spheroidal weathering.

Mineralogy of the Alkaline Rocks

Nepheline

Nepheline is an essential constituent of the syenites of the main hill at Sivamalai and the small hillocks to the west. Three varieties of the mineral have been recognised, being pale rose, grey and yellow respectively. The first two varieties are met with in the rocks of the main hill, while the yellow variety is restricted to the hillocks in the western extension. Megascopically the mineral is easily made out by its oily lustre, when fresh, and dull waxy appearance when weathered. The mineral does not exhibit any cleavage, but a subconchoidal fracture is noticed in the larger lumps. The hardness is about 6 and the specific gravity 2·58. Thin chips of the mineral are colourless and semitransparent.

Under the microscope, the mineral is characterised by its low relief and low interference colours, and is noticed to alter along the cracks into some highly polarizing sericitic material, thought to be geisickite or paragonite. Twinning has been noticed in some microsections (photomicro). Determination of the refractive index of the mineral by the immersion method gave values of $w = 1{\cdot}5252$ and $e = 1{\cdot}5225$, which are exceptionally low. Bannister (1931) has shown that the refractive index of nepheline increases with the potassium content. According to him, the number of oxygen atoms per unit cell being 32, the cell volume and presumably the refractive index are associated with the given chemical composition. Alling (1936) has however stated that the conclusion arrived at by Bannister is based on natural specimens which contain several accessory minals, and hence is different from optical data secured from synthetic preparations. A chemical analysis of the pale rose coloured nepheline, which was used for the refractive index determination shows it to be rich in soda and relatively poor in potash, which will explain the low index.

Chemical study.—A large lump of the pale rose coloured variety of the mineral was crushed and inclusions of graphite and other impurities removed with the aid of a pocket lens. The purified specimen was analysed in the usual way. The analyses of the rose and yellow varieties of the mineral from Sivamalai are given below, as also the Niggli values of these and some other nephelines.

	A	B
SiO_2 ..	43·42	43·35
Al_2O_3 ..	34·36	34·32
Fe_2O_3 ..	trace	1·02
CaO ..	1·10	0·82
K_2O ..	3·59	5·52
Na_2O ..	17·45	14·62
Loss on ignition ..	0·72	0·75
	100·69	100·40
S. G. ..	2·58	2·62

Niggli values

	si	al	fm	c	alk	k	mg
A ..	107	49	..	3	48	·12	..
B ..	110	51	2	..	46	·25	..
C ..	104	49	1	5	45	·1	·33
D ..	106	48·5	1	5·5	44	·15	·5
E ..	100	50	..	..	50	..	..

A. Pale rose nepheline, Sivamalai. Analyst: A. P. Subramaniam.
B. Yellow nepheline, Sivamalai. Analyst: T. H. Holland (*Mem. G.S.I.*, Vol. XXX, p. 187).
C. Nepheline, Monte somma
D. Nepheline, Monte somma } "Gesteins und Mineralprovinzen," Niggli (1923).
E. Theoretical composition

The analysis of the rose variety gives the formula for the mineral, $2K_2O\ 15Na_2O\ 18Al_2O_3\ 38SiO_2$.

The molecular ratio $K_2O : Na_2O$ according to the above formula is 1 : 7·5, while for the yellow variety it is 1 : 5 according to Holland. Hence the pale rose nepheline analysed appears to be different chemically from the yellow variety, confined to the hillocks west of Sivamalai.

Alkali Feldspars

Microperthites and Perthites.—The bulk of the alkali feldspars in the nepheline syenite and allied alkaline rocks is made up of varieties of microperthite and perthite. Holland has called these cryptoperthites after Brogger, who has described similar feldspars from the augite-syenites of Christiania. Following Alling (1938) three types of microperthites (hyperperthites) have been recognised in these rocks: (1) String perthite, (2) String and rod perthite, and (3) Bead perthite. Generally these microperthites exhibit a fibrous appearance under ordinary light, but under polarised light they are seen to be made up of spindle-shaped rods and thin strings. The

bead perthite referred to may be only a cross-section of the first two types. Microcline microperthite or hypoperthite, in which microcline is one of the minerals constituting the intergrowth, is also encountered in some slides.

Two types of perthites which are easily made out under low power are the patch and plume perthites, which are occasionally antiperthitic, due to development of plagioclase as host. In the patch perthites, the blebs are elongated to produce a ladder-shaped inclusion at times simulating the myrmekite structure. Plume perthite has the appearance of a feather, due to the partial interlocking of the blebs (Photo-micro).

It has been noted in many microsections that the blebs in a perthite piece are larger when adjacent to feldspar, and small when adjacent to nepheline. Thus, the nature of the adjoining minerals appears to influence the size and abundance of the rods. Alling (1938) is opposed to the view that the blebs are derived from the adjacent feldspar, in the case of perthite which lies between quartz on one side and feldspar on the other. He thinks that the bleb material came from internal sources and was prevented from developing into really bulky rods by the constriction due to the vice-like grip of the adjacent mineral-quartz. He attributes the difference in the coefficient of expansion as the probable cause of the formation of rods. The above explanation implies that the blebs were formed after the crystallization of the quartz, and that the portion of the perthite adjacent to feldspar is probably older than that close to the quartz. The above explanation is found to hold good in the case of Sivamalai perthites in relation to nepheline and moreover the reasoning is based on the assumption that the feldspar crystallized earlier than nepheline, which is found to be the case in these rocks. Penetrating blebs are also seen in some sections, the rod or film from one piece passing on to the adjoining piece of perthite, and in some cases plagioclase and even nepheline (Photo-micro).

Origin of microperthites and perthites.—Alling (1938) has brought forward three main hypotheses to explain the origin of perthites. They are (1) exsolution, (2) crystallization of trapped feldspathic fluid, and (3) hydrothermal replacement. The term 'exsolution' has been applied by Alling (1921) to the separation, into additional phases, of a supersaturated solid solution which is not in equilibrium. Kozu and Endo (1921) have furnished experimental proof of exsolution. The second hypothesis implies simultaneous crystallization of the liquid plagioclase entrapped in the cleavage planes of a crystallizing potash feldspar. Alling (1938), however, thinks that the chemical gradational nature of blebs is more suggestive of exsolution than of simultaneous crystallization. The last explanation is based on the

principle that solutions react with materials, dissolving some or all of them and depositing others in their places. Thus, if during the crystallization of igneous rocks, soda feldspar follows the orthoclase, the plagioclase would attack and partially replace the latter producing 'replacement perthite'. If, on the other hand, orthoclase crystallizes after the soda feldspars, antiperthites would be formed.

Alling has given criteria for deducing the genesis of perthites from microscopical evidence. The uniformity in shape and small size of the blebs has been suggested by him as characteristic of exsolution perthites. The distribution of rods and their avoiding margins and zones around inclusions has also been stated to be suggestive of exsolution. The above two criteria apply quite well in the case of Sivamalai perthites. Moreover the blebs tend to increase in size and abundance along the zones adjacent to neighbouring feldspars, which is suggestive of their origin by exsolution.

The typical occurrence of 'patch' and 'plume' perthites in some of the rocks, however, is suggestive of their replacement origin. The irregular shape of the blebs, their ramifying nature and their failure to maintain the same degree of uniformity in orientation, are all factors which go to suggest their replacement origin. The occurrence of bands and patches cutting the host and sending tongues into it is also considered as typical of replacement perthites. In Sivamalai rocks, the replacement type is more of an antiperthite as plagioclase is found to be the host mineral (Photo-micro). In the light of these observations it is suggested that the Sivamalai perthites are partly of exsolution type and partly of replacement type.

Holland (1901) has described the presence of opaque inclusions in the grey perthites, oriented with their longer axis lying in the clino dome. Similar inclusions have been noticed in the various types of perthites examined, and their colour may be attributed to the presence of these microscopic inclusions. Holland has indicated this by stating that the colouration of the flesh coloured perthites may be ascribed to the presence of innumerable minute platy orange coloured inclusions. These inclusions appear to be acicular hæmatite.

The chemical analysis of the grey microperthite from Sivamalai given by Holland (1901) is tabulated below together with an analysis of soda orthoclase from a Laurvikite from Norway, to bring out their close affinity.

It will be seen that the analysis agrees closely with that of soda orthoclase. The 'Ab' content as deduced from the analysis is about 52%. The optic axial angle of the microperthite was found to be 82° and the proportion of

	A	B
SiO_2 ..	64·70	62·42
Al_2O_3 ..	22·63	22·68
Fe_2O_3 ..	0·43	0·58
CaO ..	1·34	3·23
MgO ..	0·49	0·22
Na_2O ..	6·02	6·48
K_2O ..	5·86	4·42
Loss on ignition ..	0·09	..
	101·56	100·03

A. Light grey feldspar. Analyst: Dr. T. L. Walker (*Mem. G.S.I.*, Vol. XXX, p. 187).
B. Soda orthoclase from Laurvikite. Analyst: Brogger (from Johannsen's *Petrography*, Vol. IV, p. 11).

'Ab' deduced from the curve given by Spencer (1931) agrees well with that deduced from the chemical data.

Plagioclase feldspars

Microsections of rock types containing plagioclase feldspars were examined under the Universal Stage and the Anorthite content and the laws of twinning of the feldspars therein deduced after the method of Reinhard (1931). The values obtained indicated them to be oligoclase, exhibiting twinning of the Albite-Ala type. Rajagopalan (1946) has recorded the same twin law in the plagioclases of the charnockites of St. Thomas Mount. It remains to be seen whether the occurrence of an identical twin law in the charnockites and in the alkaline rocks of Sivamalai is of any genetic significance. The plagioclase feldspars are very fresh and free from alteration, another feature in common with the charnockites.

Amphiboles

Three varieties of amphibole are met with in these alkaline rocks. All of them are characterised by intense pleochroism and appear to be secondary in character.

The most prevalent type of amphibole met with in the hornblende syenite and pyroxene syenite is deeply pleochroic, with absorption in browns. A few pieces show the characteristic six-sided basal section with two sets of cleavages intersecting at 56° and 124°. Many of the pieces of the mineral are devoid of any cleavages and crystal form. A few pieces remain almost isotropic under crossed nicols. The mineral exhibits medium to strong birefringence. A few pieces which were examined under the Universal Stage gave optical constants indicating its affinity to "Girnarite", a variety of hornblende from the nepheline syenites of Mt. Girnar, described by Mathur

and Jhingran (1931). Krishnan (1926) has reported this variety of hornblende from the nepheline syenite of Mt. Girnar, referring it to barkevikite. Holland (1901) has also referred to this variety of amphibole in Sivamalai rocks as barkevikite. The optical data of the hornblende from Sivamalai and ' Girnarite ' from Mt. Girnar are given below, to bring out their affinity.

	" Girnarite "	Hornblende from Sivamalai
Scheme of pleochroism	x = deep brown	Deep brown-black
	y = straw yellow	Straw-yellow-brown
	z = yellow	Yellowish brown
	$2V = -76°$	$-60° \pm 2$
	$C \wedge Z = 9°$	$= 8°$

A second variety of amphibole with more intense pleochroism and larger optic axial angle has been observed in the hornblende-nepheline-syenites. A few pieces examined under the Universal Stage gave the following optical data:

Scheme of pleochroism	x = Yellowish brown
	y = Straw yellow-deep brown
	z = Deep brown
	$2V = -80° \pm 3$
	$C \wedge Z = 16° \pm 2$

These characters conform to those of common hornblende.

The third variety of amphibole noticed in some sections appears to be a variety of hastingsite with $C \wedge Z = 12° \pm 2$. Universal Stage determination of optical constants was not possible because of the intense absorption exhibited by the mineral. Holland has also recorded the occurrence of this variety of hornblende in some of the rocks examined by him.

Pyroxene

Aegirine-augite.—The pyroxene most commonly seen in the augite syenites is a sodic variety approximating to ægirine-augite. The mineral shows perfect cleavages and ranges in colour from emerald green to dirty yellowish green. Twin structures are very commonly seen. Holland has described the presence of gliding planes in the pyroxene and this is especially noticeable in the rocks collected north-east of Chinnamalai (·1214). Holland has stated that these pyroxenes are non-pleochroic, but this does not appear to be the case as many pieces examined showed distinct pleochroism. The mineral shows medium to strong birefringence, the interference colours ranging from yellow to pink. Optical study of several pieces of the mineral

under the Universal Stage gave the following data, indicating the mineral o belong to the ægirine-augite group.

Scheme of pleochroism	x = Light green
	y = Emerald green
	z = Bluish green
	$2V = -66° \pm 4$
	$C \wedge Z = 60°$

Hypersthene.—Pleochroic rhombic pyroxene is noted in some sections of the pyroxene syenite, and in abundance in the charnockites of the area. The mineral is easily made out by its low birefringence and characteristic pleochroism. A few pieces of the mineral on examination gave the following optical constants:

Scheme of pleochroism	x = Light green
	y = Pink green
	z = Yellowish green
	$2V = -68°$
	$C \wedge Z = 23°$

The inclined extinction often seen in hypersthenes in charnockites is noticed here also. In the pyroxene-syenites the interrelationship of ægirine-augite, hypersthene and olivine is significant, hypersthene being derived by the silicification of olivine (Photo-micro).

Titaniferous augite.—This variety of pyroxene has been noticed only in microsections of the dark hornblende nepheline syenodiorite. The mineral is easily made out by its characteristic pleochroism. The determined optical constants of the mineral are:

Scheme of pleochroism	x = Pink
	y = Greenish brown
	z = Violet
	$2V = -60°$
	$C \wedge Z = 40°$

Diopside.—This is found only in the hornblende granulites which occur as lenses within the syenite massive, and has an optic axial angle of $+60°$. The mineral is seen altering to a brown pleochroic amphibole.

Olivine.—This is an essential mineral in the pyroxene-syenites and easily made out by its high relief, characteristic cracks and strong birefringence. The optic axial angle is $+88°$. The mineral is seen to give rise to hypersthene.

Biotite.—Two varieties are met with in these rocks, a yellowish variety with intense pleochroism in browns, and a light green variety with absorption in olive greens. These are met with in the biotite-nepheline-syenite and granulitic nepheline-syenite.

Accessory minerals.—The accessory minerals met with in the syenites are calcite, graphite, zoisite, zircon, spinal, corundum, sphene, magnetite and ilmenite. Graphite occurs as disseminated flakes with lack of lustre in reflected light. Zoisite is recognised by its high relief and bluish interference colour. Zircon which is found in the pegmatoid syenites, exhibits zoning and forms pyramidal crystals of clove brown colour in the microperthites. Apatite occurs as small prisms often six-sided, while sphene is found in the more melanocratic syenites, as lozenge-shaped crystals with strong birefringence. Corundum and spinel are associated with feldspar rock, the former occurring as bluish green crystals with a flaky habit, and the latter exhibiting characteristic octahedral form. Of the ores, magnetite is found in abundance, while ilmenite is also seen in some sections. Chrysoberyl has not been noticed anywhere, though Holland has recorded their presence in the area.

Petrographic Description of the Chief Rock Types

Eight distinct types of rocks have been recognised in the alkaline series. Their petrographic descriptions follow:

Type 1: *Coarse Nepheline Syenite.*—These rocks are coarse-grained and pale bluish grey in colour. The minerals readily observed in hand specimens are feldspar, nepheline, biotite and graphite. The most abundant mineral is bluish grey feldspar with average grain size of 1 cm., showing a tabular development. The nepheline is seen as irregular rosy patches displaying oily lustre. The weathering and consequent decomposition of this type is a characteristic feature. In exposures, the rock is pitted with numerous depressions, due to weathering and removal of nepheline. Textural and mineralogical variants of the type are noticed, some being even grained with lenticles of femic minerals, simulating the " augen " structure. The specific gravity ranges from 2·58 to 2·68.

Under the microscope, thin sections of this type display hypidiomorphic texture. The feldspar is typical microperthite of the string and rod type, showing small spindle-shaped blebs. Occasionally patch perthite is also seen. Nepheline occurs as irregular patches, in between the perthite pieces. Occasionally simple twins of the mineral are noticed which is an exceptional feature. Some of the bigger pieces of nepheline carry inclusions of micro-

perthite. The only femic mineral present is biotite, occurring as lath-shaped crystals with absorption in greens. Magnetite and graphite are the accessories. The feldspars and nepheline have a turbid appearance due to alteration and also show strain shadows. The mode of a typical microsection measured with Shand's Recording Micrometer is given below :

Perthite	62·2
Nepheline	34·4
Biotite	1·7
Other minerals and graphite	1·7

This type occurs mostly in the periphery of the Sivamalai hill and the smaller hillocks to the west.

Type 2: *Aplite Syenite.*—Megascopically this variety looks like a crystalline limestone. The rock is even grained, being made up of white feldspars and subordinate amount of pale rose-coloured nepheline. It is almost devoid of femic constituents except for the lenses of biotite seen in the larger masses in the field. Occasional crystals of magnetite, corundum and zircon are noticed in some specimens. The specific gravity ranges from 2·59 to 2·64. In thin sections the rock is found to be wholly made up of perthitic feldspars. Microcline perthite and string and rod perthite are present. The perthites carry regularly oriented inclusions of a reddish opaque mineral (hæmatite ?) aligned across the line of arrangement of the blebs. Nepheline is distributed interstitially. The mode as measured on a section containing corundum is given below:

Microperthite	91·4
Nepheline	4·7
Corundum	2·9
Iron ores and zircon ..	1·0

Type 3: *Biotite-Nepheline-Syenite* (Magmatype: Nosykombitisch-Kassaitisch).—Hand specimens are rather coarse grained and greyish in colour tending to get darker with relative abundance of biotite. The biotite flakes are oriented with their longer axes parallel to the foliation direction, giving the rock a gneissic structure. Feldspars displaying pearly lustre, rose, grey and yellow coloured nepheline with vitreous lustre, biotite and graphite are easily made out in hand specimens. Rocks containing yellow nepheline are confined to the western extension of hillocks. The specific gravity ranges from 2·66 to 2·74. In thin section this type displays hypautomorphic to panidiormorphic texture. The feldspars are mostly perthitic with subordinate amount of plagioclase which has an anorthite content of

13 per cent. Nepheline is subordinate and displays creamy alteration products along the cracks, and occasionally shows poikilitic relationship with feldspars, enclosing plagioclase and perthite. A green highly pleochroic hornblende, suspected to be hastingsite, is noticed in some sections. Calcite exhibiting characteristic rhombohedral cleavages is seen in abundance, while zoisite, magnetite, zircon and graphite are also noticed. The mode for a typical section of the rock is:

Perthite	63·0
Biotite	13·0
Hornblende	9·7
Plagioclase	5·4
Nepheline	4·7
Calcite	3·3
Zoisite	0·9

This is the prevalent type in the alkaline series and a chemical analysis of the type is given later.

Type 4 *a*: *Hornblende Nepheline Syenite.*—Hand specimens of this variety have a mottled appearance, rounded patches of ferromagnesian minerals being distributed in a greyish feldspathic ground mass. Holland has described this as glomeroplasmatic structure. The specific gravity of these rocks varies from 2·65 to 2·75. The feldspars have a pearly lustre and are greyish white in colour. A few colourless grains of nepheline are noticed all over the rock. In thin section these rocks display a variety of textures from granitoid to granophyric. The chief salic constituent is perthite, a portion being antiperthite. The plagioclase has an anorthite content of 13% and is subordinate to the perthite. Nepheline is sparsely distributed, mostly interstitially. Hornblende and biotite which make up the femic element in the rock are both deeply pleochroic. The amphibole here is the brown variety approaching "Girnarite". Calcite, zoisite, magnetite and graphite occur in most of the thin sections examined.

Type 4 *b*: *Pink Hornblende Nepheline Syenite.*—This type, found on the western flank and periphery of the main mass, is a variant of the previous type and is characterised by the pink colour of the feldspars, and the rough foliation exhibited. Little nepheline is seen on hand specimens but biotite and hornblende are easily made out. The specific gravity of this variety ranges from 2·65 to 2·70. In thin section the type displays hypidiomorphic texture. The feldspars are hypoperthite, film perthite and oligoclase, the last forming rims around the perthites. Biotite and hornblende are similar to those in the previous type and the femic minerals tend to cluster together.

Calcite, zoisite, magnetite, zircon and graphite occur as accessories. The mode for type 4 is given below:

Feldspars	75·6
Biotite	8·7
Hornblende	8·7
Nepheline	4·2
Calcite	1·4
Zoisite + apatite + ores ..	0·8

Type 5: *Hornblende Nepheline Syenodiorite* (Magma type: Mugearitisch).—This type is found on the western flank of Sivamalai, being distinctly melanocratic and having a high specific gravity (3·0 to 3·1). The rock has a granulitic texture, small platy crystals of feldspar being intermingled with nepheline, hornblende forming the only other perceptible component. In thin section the rock presents a panidiomorphic texture, the feldspars having almost rounded outlines. The feldspars are mostly basic oligoclase (An 18%) and orthoclase, perthite being conspicuously absent. Nepheline is distributed interstitially. The chief femic constituent is a brown hornblende with intense absorption and it appears to be secondary. Pleochroic titaniferous augite showing absorption in pinks is present in the section, and is noticed to give rise to secondary hornblende. Calcite, graphite, zircon, magnetite, apatite and sphene form the accessory minerals, the last named being conspicuous in its being present only in this type. Holland has described this type as a " Basic lens with basaltic hornblende ". The mode for a typical thin section is given below:

Feldspars (Or + Plag)	48·2
Hornblende	32·0
Nepheline	11·3
Titan. augite	2·2
Calcite	1·5
Sphene	2·3
Iron ores + zoisite + graphite ..	2·5

A chemical analysis of this type has been made and will be discussed in a later section.

Type 6 *a*: *Granulitic Nepheline Syenite.*—The rock is grey coloured with an even-grained texture. Biotite and graphite are easily made out in the specimen, while grey coloured nepheline and feldspar are recognised with a pocket lens. The specific gravity ranges from 2·65 to 2·70. In thin section the rock shows a granulitic texture and is seen to be made up of microperthite and nepheline with biotite as the only femic constituent.

Perthite is of the string and rod type, and is occasionally bordered by a film of microcline. Nepheline occurs as irregular patches and is much cracked up. Biotite is strongly pleochroic in olive greens, and is roughly oriented along the foliation direction. Magnetite, zoisite and apatite occur as accessories. The mode for the type measured on a typical thin section is:

Perthite	48·1
Microcline	6·6
Nepheline	23·9
Biotite	13·8
Magnetite + zoisite + apatite + graphite	7·5

Type 6 b: *Granulitic Nepheline Syenite with Plagioclase and Microcline.*—This type is noticed on the northern slope of Sivamalai and in the Kosamalai ENE of Hanuman temple. Hand specimens are very similar to the previous type but show slight foliation. In thin section the rock shows an appreciable amount of plagioclase with 35% anorthite, and subordinate amount of microcline. Biotite, magnetite, apatite and graphite occur as in the previous type.

Type 7: *Aegirine Augite Syenite* (*Magma type*: *Melanatronsyenitisch*).—This type is found in the Chinnamalai (1,214 ft.) and a number of knolls beyond it. It is melanocratic, a freshly broken surface presenting a beautiful greenish black tint. This is due to the abundance of coloured perthite which constitutes the bulk of the rock. Occasional crystals of pyroxene and grains of olivine are made out with a pocket lens. Textural variation is not so marked as in the nepheline syenites, and the specific gravity ranges from 2·75 to 2·80. In thin section these rocks show a hypidiomorphic texture. The feldspar is mostly microperthite with occasional films of hypoperthite. Occasionally a subordinate amount of plagioclase with 13% anorthite is noticed. Nepheline is conspicuous by its absence and staining was resorted to on a few sections to verify its absence. The chief femic constituent is a beautiful green pyroxene, pleochroic in emerald and bluish greens, and is identified as ægirine-augite from its optical constants. Olivine and hypersthene are noticed closely associated with and sometimes partially enveloping the green pyroxene. Brown pleochroic hornblende occurs as an alteration product. The accessory minerals are zoisite, magnetite and apatite. The mode for a typical section is given below:

Perthite + plagioclase	81·7
Pyroxenes	8·0
Olivine	7·0
Iron ores + apatite	3·0

The tendency of the ferromagnesian minerals to cluster and form aggregates is marked in this variety and explains the leucocratic nature of the mode recorded above.

Type 8: *Microperthitite.*—This type shows enormous textural and colour variations and is wholly composed of perthitic feldspar. The colour of the rock ranges from pink to mauve and grey to milk white. The size of the crystals ranges from 1 cm. to as much as a foot. At places the large masses of this variety carry corundum, spinal and zircon. The specific gravity is about 2·6. In thin section the rock is made up of string and rod perthite with opaque inclusions lying across the orientation of the blebs. This type forms the periphery of the alkaline intrusive and also forms contemporaneous veins on the hill. The nomenclature adopted is in view of the monomineralic nature of the rock.

Chemical analyses of Sivamalai alkaline rocks

	A	B	C	D
SiO_2 ..	52·71	48·46	55·65	55·68
Al_2O_3 ..	20·41	21·42	16·47	23·81
Fe_2O_3 ..	1·42	2·19	4·86	4·84
FeO ..	6·03	8·27	7·01	
MgO ..	1·83	4·46	2·48	0·65
CaO ..	5·04	5·20	2·58	1·69
Na_2O ..	7·21	5·64	6·83	9·23
K_2O ..	4·25	1·45	3·74	5·16
TiO_2 ..	0·51	1·61	0·79	..
P_2O_5 ..	tr.	0·38	..	..
H_2O^+ } ..	1·24	1·16	0·16	0·34
H_2O^- }				
Graphite ..	..	..	..	0·58
Total ..	100·65	100·24	100·57	101·98
Specific gravity ..	2·72	3·00	2·80	2·59

Niggli, Q.L.M. and π values

si ..	147	118	160	171
al ..	34	31	28	43
fm ..	24	40	38	14
c ..	15	13	8	6
alk ..	27	16	26	37
k ..	·30	·15	·26	·27
mg ..	·28	·41	·28	·21
Q ..	28	26·1	25	25·2
L ..	50	49·2	59	68·5
M ..	22	25	16	6·3
π ..	·10	·28	·04	·06

	A	B	C
		Basis Composition	
Kp ..	14·7	5·3	12·8
Ne ..	37·8	30·0	36·1
Cal ..	6·3	13·7	2·2
Cs ..	4·1	..	2·7
Fo ..	3·7	9·2	5·1
Fa ..	6·8	10·6	8·0
Fs ..	1·5	2·3	5·1
Cp ..	..	0·8	..
Q ..	21·8	25·0	27·5
Ru ..	0·3	1·1	0·5
C ..	..	2·1	..
		Kata Molecular norm	
Or ..	24·5	8·8	21·3
Ab ..	26·1	31·2	47·8
An ..	22·1	22·8	3·7
Ne ..	10·5	11·2	7·4
Fo ..	3·7	9·2	5·1
Fa ..	6·1	9·5	5·5
Cp ..	..	0·8	..
Ru ..	..	1·1	0·5
Mt ..	1·5	2·3	5·1
Wo ..	4·9	..	3·6
Tn ..	0·9	..	..
Sill ..	..	3·1	..
		C. I. P. W. norm	
Or ..	25·0	8·34	21·7
Ab ..	21·5	34·06	44·5
An ..	10·8	25·02	3·6
Ne ..	21·3	7·1	7·1
Ol ..	5·5	17·09	7·2
Di ..	12·0	..	7·6
C ..	..	1·53	..
Mt ..	2·0	3·04	7·2
Il ..	0·9	3·25	1·5
Ap ..	..	1·01	..
		Modes	
Perthite ..	63·0	..	81·7
Plagioclase + orthoclase ..	5·4	48·2	..
Nepheline ..	4·7	11·3	..
Hornblende ..	9·7	32·0	..
Pyroxenes ..	..	2·2†	8·0‡
Biotite ..	13·0	..	..
Olivine ..	..	..	7·0
Calcite ..	3·3	1·5	..
Zoisite ..	0·9	2·5	3·0
Apatite ..			
Ores ..			
Graphite ..			
Sphene ..		2·3	..

† Titaniferous augite. ‡ Aegirine augite + Hypersthene.

A. Foliated biotite nepheline syenite, Sivamalai hill eastern slopes. Analyst: A. P. Subramaniam.

B. Hornblende nepheline syenodiorite, Sivamalai hill western slopes. Analyst: A. P. Subramaniam.

C. Aegirine augite syenite, N.E. slope of Sivamalai, south of pagoda. Analyst: A. P. Subramaniam.

D. Even grained nepheline syenite, Sivamalai hill. Analyst: Dr. T. L. Walker, *Mem. G.S.I.*, Vol. XXX, p. 181.

The tabular statement above gives the results of chemical analysis of type (3), (5) and (7) and that of even grained nepheline syenite given by Holland together with their Niggli values. The basis composition, C.I.P.W. and kata molecular norms and modes of types (3), (5) and (7) are also tabulated. In view of the limited chemical data it will be premature to go into a discussion on the petrochemical aspects of these alkaline rocks. However, when plotted on the Q.L.M. and πk diagrams, the analyses bring out their alkaline nature, and their affinity to the Atlantic suite.

Mode and Norm

Type 3: *Biotite-Nepheline-Syenite* (Magma type: Nosykombitisch-Kassaitisch). The total modal feldspar and feldspathoid is about 73%. Taking this from the norm, Or + Ab + Ne + An (partly), the proportion will be 24 Or, 26 Ab, 22 Ne and 1 An. Allotting the proper proportion of Ab molecule to An and removing the nepheline molecule in proportion to the modal value, the proportion remaining for perthite comes to 24·5 Or, 22 Ab, and 17·8 Ne, which means that perthite carries unsaturated soda molecule. A part of the lime goes into combination with hornblende and some is present as calcite. The hornblende as deduced from the norm appears to be rich in lime and alumina, conforming to the optical data.

Type 5: *Dark Hornblende-Nepheline-Syenodiorite* (Magma type: Mugearitisch).—Comparing the mode with the norm it is seen that the modal feldspar comes to 48 % (Or + plagioclase). Taking this amount from the norm to make up 48%, the anorthite content of the normative plagioclase will be near about the determined value, *viz.*, 18%. The rest of the normative anorthite is apparently present in the femic mineral hornblende, which optically appears to be a lime-rich variety. The lime of part of the normative anorthite is in the form of calcite and of sphene (with TiO_2). The Al thus liberated is in combination with the hornblende. The normative sillimanite reckoned as corundum in the C.I.P.W. norm is also in the hornblende. The normative and modal nepheline are in agreement. Streckeisen (1938) has offered a similar explanation for the leucocratic nature of the norm of a melanocratic rock.

Type 7: *Aegirine-Augite-Syenite* (Magma type: Melanatronsyenitisch).—Here the modal value does not compare favourably with the normative value since the coarse glomeroplamatic structure resulting from the clustering of the femic minerals in groups does not favour micrometry.

THE Q-L-M-DIAGRAM

The behaviour of silica during the progress of crystallization differentiation can be observed in the Q.L.M. diagram of Niggli. In this diagram the three corners of an equilateral triangle represent three groups of components, Q = quartz, L = leucocratic base molecules (Kp + Ne + Cal) and M = (Melanocratic) base molecules (Fo + Fa + Fs) the composition of any particular rock being represented by a point within the diagram. In the diagram 'P' on the side QM and F on the side QL represent the position of pyroxenes and feldspars respectively. M representing the unsaturated femic minerals takes a certain amount of Q for the formation of pyroxene involving the following reaction:

$$\text{Mg Mg SiO}_4 + \text{SiO}_2 = 2\ \text{Mg SiO}_3$$

i.e., 3 Fo + 1Q = 4 En

Therefore 3 M + 1 Q = 4 P

or

75 M + 25 Q = 100 P

Similarly for feldspars

$$\text{K Al SiO}_4 + 2\ \text{SiO}_2 = \text{K Al Si}_3\text{O}_8$$

i.e., 3 Kp + 2 Q = 5 Or

Therefore 3 L + 2 Q = 5 F

or

60 L + 40 Q = 100 F

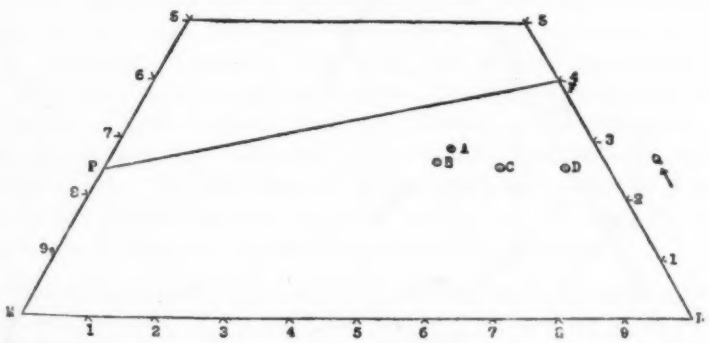

FIG. 3. *Q.L.M.* Diagram

Since the feldspars and pyroxene represent the fully saturated products of crystallization, the line PF which is the saturation line divides the field into two zones, the unsaturated area PFLM and the saturated area PFQ. It can be seen from the diagram that the rocks of the Sivamalai area arrange themselves well below the saturation line, in a zone which according to Niggli (1938) is characteristic of alkaline rocks.

πk Diagram

Here the value gives the proportion of normative anorthite in the total normative feldspars and is expressed as

$$\pi = \frac{\text{Cal}}{\text{Kp} + \text{Ne} + \text{Cal}}.$$

The relationship of the three basis molecules are brought out in a trilinear diagram where the values Kp, Ne and Cal occupy the three corners of the triangle. The value of 'k' is measured on the side Kp-Ne with 100% at Kp; while π is measured either alongside Ne–Cal or Kp–Cal with 100% at Cal. Values of π are represented along a line parallel to the side Kp–Ne,

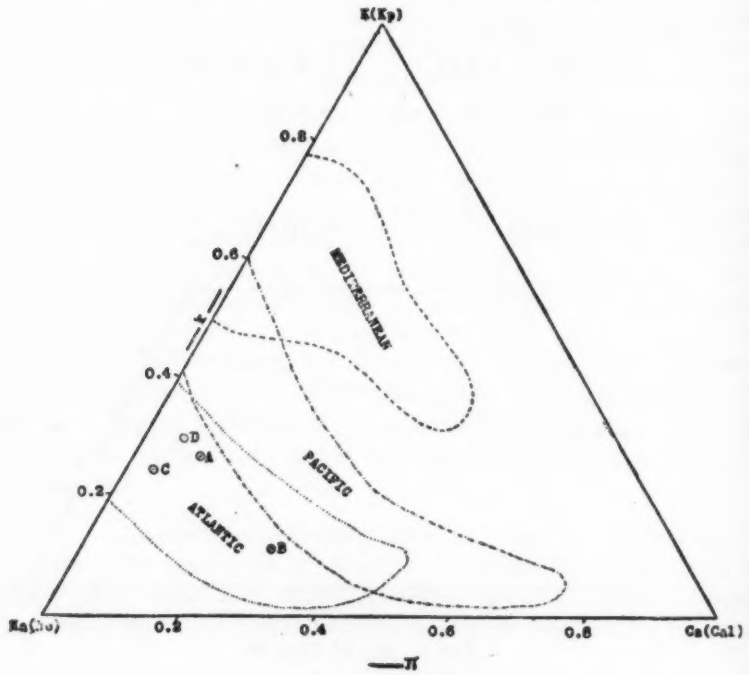

Fig. 4. π–k diagram

while lines joining the Cal corner from Kp–Ne side will give particular values of 'k'. The Atlantic affinity of the alkaline rocks is shown out in the diagram where the three fields constructed by Niggli (1938) for the three petrographic suites are incorporated in a simplified form. The alkaline rocks of Sivamalai fall within the Atlantic field.

Primary Calcite in the Alkaline Rocks.—The presence of calcite as an undoubted primary constituent in these rocks is a feature in support of their syntectic origin. Heron (1924) records the occurrence of primary calcite in the Sodalite Nepheline Syenites of Kishengarh. Daly ascribes the presence of calcite to absorption of limestone by the igneous rocks, while Shand (1922) attributes the presence of calcite and other like minerals in Nepheline Syenites to the addition of lime to the freezing magma by circulating fluids. Workman (1911) thinks that an alkaline magma in which the silica present is thoroughly saturated with bases, may quite well absorb calcite without leading to the dissociation of the mineral. Bowen (1924) however prefers to consider calcite to be of hydrothermal origin. Streckeisen (1938) considers primary calcite to be formed as a result of the CO_2 content of the magma combining with lime. Osborne (1930) is of the opinion that calcite may be residual from limestone or may be pyrogenetic. The calcite in the Sivamalai nepheline syenites appears to be primary in nature and may have resulted from absorption of limestone by the magma. The pyroxene syenites however do not contain any calcite or graphite.

Origin of graphite.—Holland considered graphite in the nepheline syenites to be as much an igneous mineral as nepheline in view of its exhibiting the phenomenon of sprouting, regarded as characteristic of graphite crystallized from fusion. Winchell (1911) assumed that the carbon in the magma may unite with H_2O at a temperature of over 700° C. forming CO and CO_2 or both, and hydrogen, the reaction being reversible at 500° C. with the formation of graphite and water. In Russian occurrences (Min. Abstr. 1926–28) graphite is supposed to be a decomposition product formed by the action of gases from the nepheline syenites on the carbonates. The graphite in the Sivamalai rocks may have resulted from both the reactions mentioned above. The development of amphibole and biotite to a marked degree in the graphite-bearing nepheline syenites of the main hill is suggestive of the presence of hyperfusibles during crystallization.

Origin of Pegmatitic Syenites.—The development of coarse pegmatite veins of feldspar rock in the main hill and in the periphery of the alkaline rocks may be ascribed to assimilation, the inclusions and marginal country rocks accelerating crystallization near about. This is in conformity with

Bowen's (1922) suggestion that inclusions near the low temperature end of the solid solution series with which the magma is saturated will result in increased amount of liquid, leading to a pegmatitic condition.

Origin of foliation.—The foliation noted in the syenites of this area is a feature not commonly associated with eruptives. Banding in eruptives is attributed to differentiation by some, while Alling (1924) and a few others ascribe it to syntexis. Alling has stated that the darker constituents are due to the almost complete digestion of foreign bodies, and these in gneissic rocks are in truth the residue of included rocks. Osborne (1930) states that banding can be due to an alternation of dark and light bands, the salic bands being injected in the darker femic portion. The banding observed in the nepheline syenites may be due to syntexis accentuated by the reorientation of the femic minerals during subsequent regional metamorphism. The granulation, angular nature of mineral constituents and the strain shadows observed in many microsections are indicative of the stress undergone by these rocks.

Genesis of the alkaline suite.—The present study has not helped in deducing the genesis of these rocks, but the occurrence of xenoliths of hornblende-dipside-granulites, presence of calcite in the nepheline syenites and the close association of charnockites, pyroxene syenite and nepheline syenites in the area are suggestive of the probable syntectic evolution of these rocks. The study of these rocks from this angle will have to be taken up in order to establish the mode of origin of these highly interesting group of rocks.

In conclusion, the author wishes to acknowledge his indebtedness to Dr. M. S. Krishnan for many helpful suggestions in the preparation of this paper and for a critical reading of the same.

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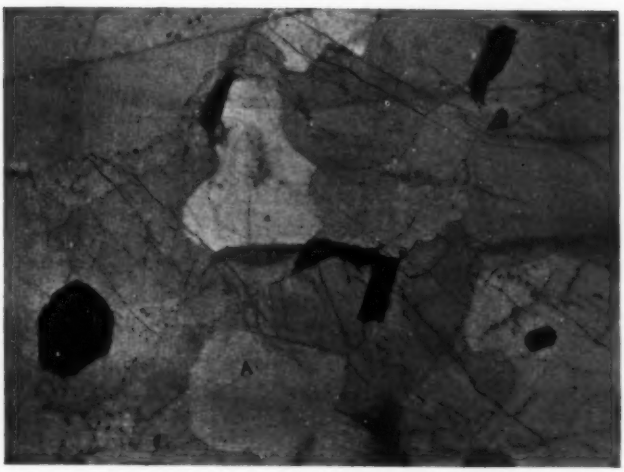

FIG. 1

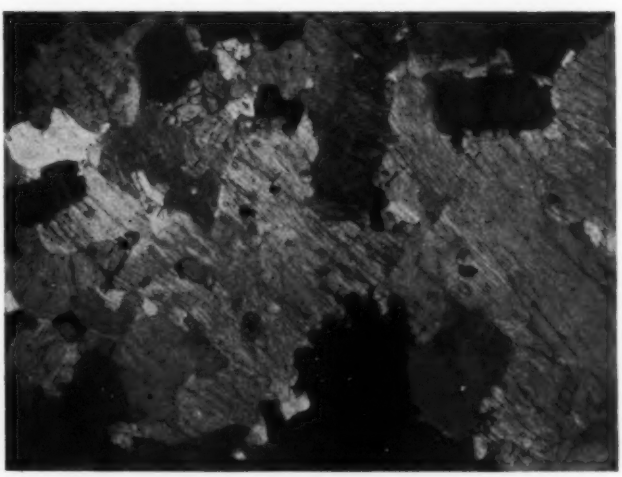

FIG. 2

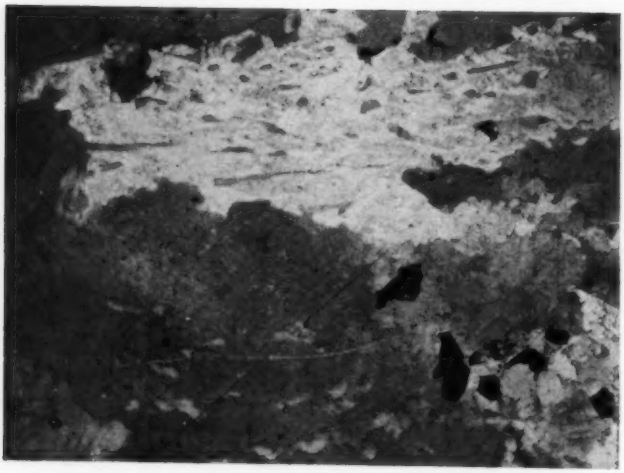

FIG. 3

FIG. 4

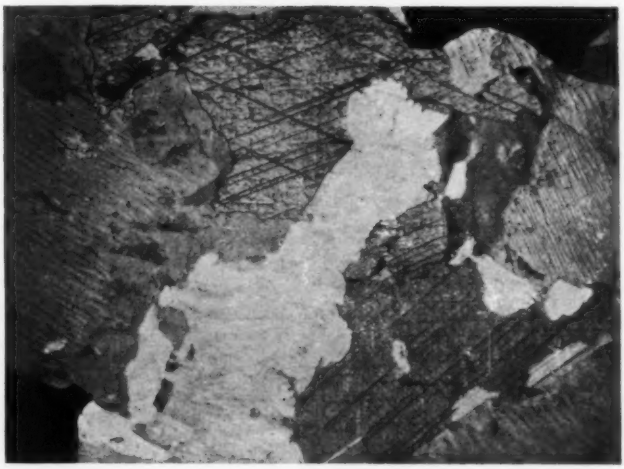

FIG. 5

FIG. 6

FIG. 7

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PHOTOMICROGRAPHS

1. Twin crystal of nepheline in biotite nepheline syenite. (A) — nepheline. ×40.
2. Penetrating blebs of microperthite in granulitic nepheline syenite. ×40.
3. Antiperthite in aplite syenite. ×40.
4. Plume perthite in nepheline syenite. × 40.
5. Primary calcite in nepheline syenite. ×40.
6. Pyroxene syenite, showing interrelationship of ægirine. Augite = (A), olivine = (B), and hypersthene = (C). Aegirine augite is encircled by olivine and hypersthene developed along the margin of olivine. ×40.
7. Hornblende-diopside-granulite. Diopside = (A), altering to hornblende = (B). ×40.

REACTIONS OF FATIGUED UNSTRIATED MUSCLE AND THEIR RELATION TO THOSE OF CARDIAC MUSCLE

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One of the properties of the heart muscle when compared to unstriated muscle is that the former shows powerful rhythmic contractions; though unstriated muscle shows spontaneous contractions, these are much smaller than a powerful contraction produced by other forms of stimulation such as by electric current. So to compare the two kinds of muscle, the unstriated muscle should also be similarly rhythmically stimulated. This has been done in the present research and the reactions of fatigued unstriated muscle have been studied. There is no doubt that the cardiac muscle is normally fatigued as shown by the fact that the contraction following a compensatory pause may be bigger than normal and also if the heart rate is slowed, such as by vagus stimulation, the size of the contractions increases.

Experimental

Frog's stomach muscle (Singh, 1939), dog's stomach muscle (Singh, 1940) and fowl's gut muscle (Singh, Singh and Muthana, 1947) were stimulated once a minute by alternating current, 12 volts for 10 seconds.

Results

When unstriated muscle is frequently stimulated, fatigue is very rapid; when the contractions assume constant height after one to five stimulations, the response may be 5–10 per cent. of the first one. After fatigue, the contractions may begin to increase (Fig. 1). This increase is due to shortage of oxygen supply due to lag in diffusion of gas into the muscle, as it has all the properties of the asphyxial increase in excitability. This secondary increase in excitability is not the same as the beneficial effect of contraction, as the latter is produced when the muscle is stimulated once in 15–20 minutes; the possibility of asphyxia is thereby excluded.

The above experiments suggest the necessity of a rich blood supply to a rhythmically contracting tissue such as the cardiac muscle. It is known that cardiac muscle has a very rich blood supply and the presence of sarcolemma is problematical. If unstriated muscle, which is in a state of persistent

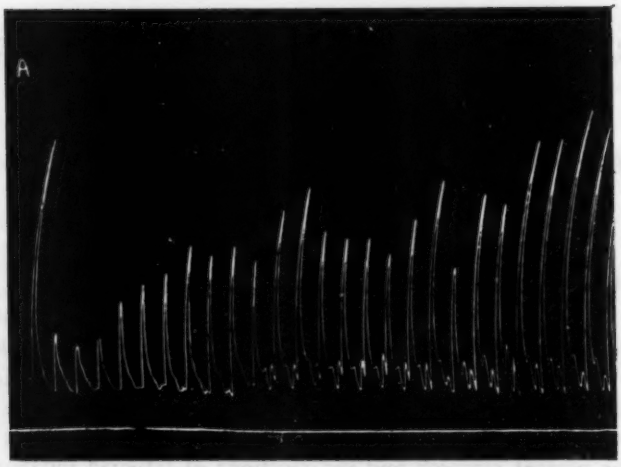

FIG. 1. Frog's stomach muscle. Stimulation by alternating current 8 volts for 10 seconds per minute

contraction, does not possess such a blood supply; then it suggests that tone does not require much oxygen.

Frequent stimulation causes reduction of tone, which again increases when the stimulation is stopped (Fig. 2). This may account for the absence of tone in heart muscle, as tone is antagonistic to twitch, the former will interfere with proper functioning of the heart. After cessation of stimulation, tone may increase temporarilly above normal suggesting the opening of reserve metabolic channels during increased activity, as happens in asphyxia (Singhe, 1949).

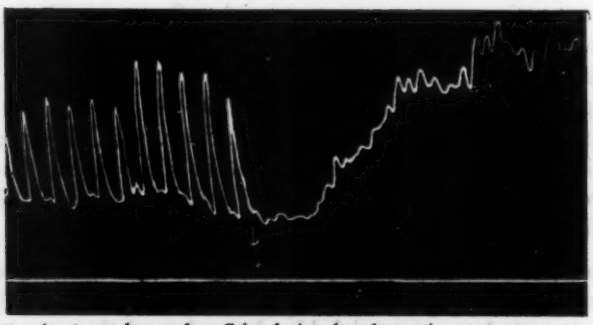

FIG. 2. Frog's stomach muscle. Stimulation by alternating current up to the arrow

The diminution of tone on frequent stimulation resembles the diminution of negative and increase of positive potential in nerve. Changes in

tone following a single contraction of unstriated muscle resemble those in nerve as regards potential. The twitch represents the spike potential and the slow decline the negative after-potential. This is followed by decrease of tone before return to normal and this corresponds to the positive after-potential in nerve. From this it appears that nerve is normally in a state of "tone".

Tone producing substances relieve fatigue (Figs. 3, 4). This action is temporary as later on they diminish excitability. The preliminary phase of increased excitability may be absent. The effect of tone producing substances in relieving fatigue is similar to their effect in relieving asphyxial arrest. The above action resembles the effect of barium on heart muscle and the effect of adrenaline and Orbeli effect on skeletal muscle.

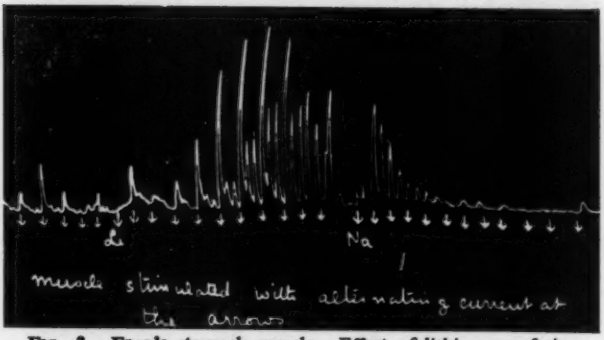

FIG. 3. Frog's stomach muscle. Effect of lithium on fatigue

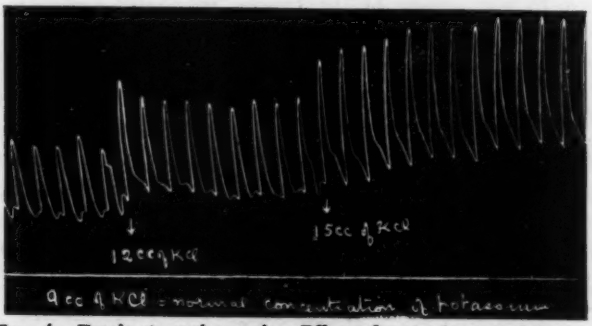

FIG. 4. Frog's stomach muscle. Effect of potassium on fatigue

The sensitivity to chemical stimulation decreases, so that the muscle may become inexcitable to potassium and acetylcholine, the excitatory effect may be replaced by an inhibitory one. Thus the reactions of fatigued unstriated muscle may stimulate those of the heart muscle. The absence of

tone and the action of potassium and acetylcholine in heart muscle are therefore related to its superior power of rhythmic contraction.

Fatigued unstriated muscle requires more calcium than normally for the response to alternating current (Fig. 5).

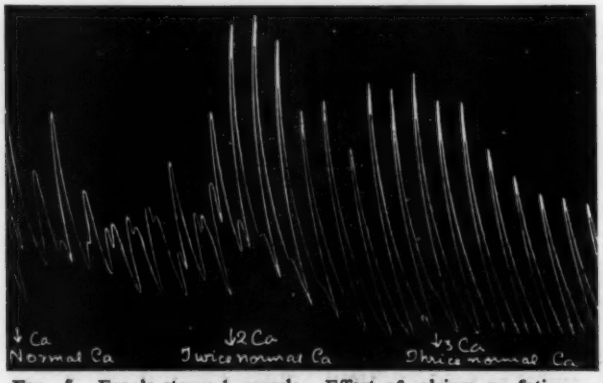

FIG. 5. Frog's stomach muscle. Effect of calcium on fatigue

In fatigued unstriated muscle, the inhibitory action of adrenaline decreases, and its stimulatory action may increase, thus approaching its action on cardiac muscle. This also resembles the effect of adrenaline and the Orbeli effect on skeletal muscle.

DISCUSSION

Some of the differences between cardiac and smooth muscles can be accounted for by the power of the former to contract rhythmically. The most important difference between the two muscles is the absence of tone in the former. This increases the efficiency of cardiac muscle as a rhythmically contracting muscle. Lack of tone implies diminished sensitivity to chemical stimulation. In unstriated muscle, when sensitivity to chemical stimulation decreases, then some substances which usually cause tonic contraction, now increase the response to alternating current. This explains the action of adrenaline and barium on the heart muscle. Other substances may produce inhibition in unstriated muscle; this explains the action of potassium and acetylcholine on the cardiac muscle.

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STUDIES IN STORAGE OF GUR—I

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I. Introduction

The season for manufacture of gur is confined to the cold months, extending from November to the middle of April and the product has to be stored for consumption during the remaining part of the year. The conditions of storage in different parts of the country are described at length in the report on the marketing of sugar in India and Burma (1943) where the total costs of carrying over and seasonal rise in prices are estimated for different places under existing conditions, showing a small margin of profit for the stockist. It is, however, an admitted fact that gur under storage is liable to undergo great losses in quality as well as quantity and the problem of storage is one of paramount importance. In the eastern districts of India which receive a heavy rainfall in the months of June, July, August and September and experience humidities of a very high order, storage of gur presents serious difficulties and the stockist has to encounter considerable deterioration and loss of material. Deterioration is brought about by the action of micro-organisms causing inversion of sucrose and formation of invert sugar. Although sucrose itself is a preservative and products containing sucrose of

the order of 75% are not liable to easy deterioration, the absorption of moisture on the surface due to the presence of invert sugar and highly hygroscopic mineral salts brings about a great reduction in the surface concentration of sucrose, thus taking away the immunity from action of micro-organisms. The surface being once affected and inversion having started, the invert sugar formed attracts large quantities of moisture when the deterioration in progress on the surface layers is greatly accelerated and rapidly spreads through the body of the gur. Attendant with the increasing amount of invert sugar, there is quick absorption of moisture as a result of which the gur becomes soft and sticky, often liquefying completely and being entirely lost. The process of moisture absorption and inversion are accompanied by gradual fermentation which imparts an unpleasant taste and smell to the gur. Over and above these, the gur darkens in colour obviously on account of some of the products of decomposition being dark and the texture is adversely affected in that the gur loses its crystalline nature, becoming amorphous and plastic. The growth of fungus and worms is also met with and the development of an acid or bitter taste is quite common. The extent of loss suffered in storage is thus quite clear and the agency causing it is the high atmospheric moisture from which mainly the product in storage must be protected.

Different methods of gur storage are in vogue in different parts of the country, an account of which may be found in the report on the marketing of sugar (*loc. cit.*). For example, in parts of Central India, Bombay and Hyderabad, gur lumps are generally stored in cotton seed and in the moist eastern districts of the United Provinces, Bihar and Bengal, a lining of bhusa (wheat straw) is used for this purpose. It is, however, a matter of common experience that in the very moist East Indian conditions, storage materials of this kind do not provide a satisfactory solution of the problem. The weather conditions prevailing in these parts of the country will be seen from Table I, giving the average figures over a large number of years for rainfall and humidity at Pusa in Bihar. The concentration of rainfall in the monsoon months is remarkable inasmuch as 39·24 inches of rainfall occurs in the period June to September out of the total figure of 46·00 inches for the whole year and the humidity at this time of the year is in the vicinity of 80–85 per cent. These facts clearly indicate the highly moist conditions prevailing in this season and the necessity for a very effective method of storage need hardly be emphasised.

Apart from rough qualitative observations, very little scientific data are available on the changes occurring in the physical and chemical properties of gur under different conditions of storage. Scientific work of any

kind in relation to gur is singularly lacking and whatever is available in the literature is mainly confined to studies in the intrinsic properties of gur (Varahalu, 1937, 1938, 1939). A few observations on behaviour under storage are, however, available in the investigations conducted at the Imperial Institute of Sugar Technology, Cawnpore (Rao, Kripashankar and Gundu Rao, 1933) but these are by no means of an exhaustive nature. It was, therefore, considered to be of interest to examine the nature of changes in the physical and chemical properties of gur under storage in different blankets as also in exposed condition, thus ascertaining at the same time the characteristics associated with gur in different stages of deterioration. This piece of work, was, therefore, undertaken with the two-fold object of:

(1) devising a satisfactory method of gur storage,

(2) studying the changes in physical and chemical attributes of gur under different conditions of storage, and ascertaining at the same time the characteristics associated with gur at different stages of deterioration.

II. Choice of Storage Methods

A blanket for gur storage must be of such material as would prevent the least access of moisture to the product stored in it. The commonly used lining of wheat bhusa does not fulfil this condition and as such it gives unsatisfactory results. An effective storage blanket must arrest all the moisture in itself and thus prevent its reaching the stored material. This may be accomplished by a substance possessing a high adsorption capacity or hygroscopicity. The well-known adsorptive properties of powdered charcoal would suggest its possible usefulness as a storage blanket of the first type. As an hygroscopic material, one would think of ash which can absorb large quantities of moisture by virtue of the highly soluble mineral salts contained in it. The gur furnace provides a plentiful source of this material and it was, therefore, expected to prove a very suitable blanket for gur storage. In these experiments charcoal powder and furnace ash were examined with respect to their values as storage blanket in comparison to the country method of bhusa storage as also storage under exposed conditions.

III. Important Physical and Chemical Attributes of Gur

Besides the general features, such as hardness, colour, texture and taste, the following physical and chemical attributes were considered important in determining gur quality and changes in these have been examined under different storage conditions.

1. *Sucrose content* (Polarisation).—Needless to say, a high sucrose content characterises good quality.

2. *Glucose.*—This term is used in a wide sense to denote reducing sugars. As these substances promote a non-crystalline texture and by virtue of their hygroscopic nature attract moisture and accelerate decomposition, gur of good quality would be associated with low glucose content.

3. *Moisture.*—A large moisture per cent. in gur means a soft and sticky character as well as liability to easy deterioration and is therefore a sign of bad quality.

4. *Acidity.*—Development of acidity proceeds with deterioration and smaller values for acidity would thus indicate better quality.

5. *Intensity of colour.*—As light coloured gur is preferred to a dark product, a measurement of the intensity of colour of standard gur solutions would provide a reliable gradation in this respect.

6. *Concentration of suspended impurities.*—A smaller concentration of suspended impurities would denote greater purity of the product and a gradation in this respect may be accomplished by measuring the turbidities of standard solutions.

7. *Pore space.*—Openness in texture of gur is a desirable criterion, a closed texture being associated with amorphous character. A classification with regard to this criterion is available in the measurement of pore space.

IV. Experimental Procedure

A number of gur samples (from different varieties of cane and prepared by different juice treatments) were stored by the three methods, *viz.*, in furnace ash, powdered charcoal and wheat bhusa. A few samples were also kept on open racks completely exposed to the atmosphere. All samples were examined before storage in respect of their general features and analysed for the different quantitative criteria. This was periodically repeated (in course of storage) in the beginning of every month from May to October. Representative samples of whole blocks were always used in the determinations and the experiments were conducted over three successive years. The blocks were always of the same size and shape to ensure comparability of the results.

(i) *Preservation of gur in storage blanket.*—Gur is preserved inside the storage blankets in the following manner:

A thick layer of the storage material (say furnace ash) is spread at the bottom of a basket and blocks of gur are arranged over it. These are well

covered on all sides with liberal quantities of the material which is tightly packed so as to provide a compact envelop on all sides. Another heavy layer of storage material is placed on the gur layer and more blocks of gur kept over it in the same manner. Alternate layers of gur and the storage material are thus arranged, ending in a heavy covering of the latter on top.

(ii) *Analytical methods.*—Sucrose is determined in ½ normal solutions of gur (65 gm. in 500 c.c.) by the usual polarisation method, the results being expressed as per cent. on weight of gur.

Glucose is estimated by the potassium ferricynide titration method of Khanna and Sen (1938) with clarified and deleaded solutions of the concentration stated before and the results are calculated as per cent. on gur weight.

Moisture is determined by drying weighed samples of gur in an air oven at 60–70° C. for 8 hours.

Acidity is determined by titrating standard solutions of gur with N/50 caustic soda solution, using phenolphthalein as indicator and the results are written as milliequivalents acid per 100 gm. of gur.

Intensity of colour is measured for ¼-normal solutions (6.5 gm. in 100 c.c.) by means of the Klett Summerson Photoelectric Colorimeter after filtration through a double layer of filter-paper. The Colorimeter is set to distilled water at zero and the scale readings of the instrument recorded for different solutions and taken as comparative indices of the depth of colour.

For gradation of samples in respect of concentration of suspended impurities, measurements of the turbidity of ¼-normal solutions were carried out by means of the Photoelectric Colorimeter. The ratio between the scale readings of this instrument for an unfiltered solution and for the same solution filtered through a double layer of filter-paper is designated turbidity which is regarded as a measure of the concentration of suspended impurities.

The method of determining pore space consists in soaking weighed samples of gur (approximately 3 mm. cubes) in kerosene for 24 hours when it is considered to have filled the pores. The cubes are weighed after removing the kerosene sticking to the exterior by means of a filter-paper, the latter operation taking about a minute. The weight of kerosene absorbed divided by the specific gravity of kerosene gives the volume occupied by

the kerosene or the pore space. The results are expressed in c.c. per 100 gm. of gur. The cubes are removed by means of a sampler knife as closely as possible from the central region of the bulk of the gur block, thus truly representing conditions in the heart of the sucrose core. This, together with the fact that the original gur blocks are of identical size and shape, sufficiently ensures the representative nature of the samples and comparability of the values.

V. Experimental Results

The data have been incorporated in the different tables. Table I gives average data for rainfall and humidity at Pusa for a large number of years and the general features in these respects have already been referred to. Table II relates to typical observations on the behaviour of gur under different storage conditions. Tables III, IV and V provide a statistical comparison of ash, bhusa and powdered charcoal as storage blankets. In Table VI is examined the question of varietal differences in keeping quality and in Table VII a consideration of the effect of juice treatments on the same has been included. Table VIII gives some observations on weight losses in storage.

Plates XIV and XV furnish a comparative picture of the condition of gur samples after storage through the monsoon in different blankets.

Table I

Showing rainfall and humidity data for Pusa

(Average for the period 1932–45)

Month	Average rainfall (inches)	Mean humidity per cent.
January ..	0·67	79·2
February ..	0·82	73·9
March ..	0·34	55·9
April ..	0·42	52·9
May ..	1·80	66·0
June ..	7·08	76·2
July ..	9·88	83·2
August ..	12·32	85·9
September ..	9·96	83·3
October ..	2·40	77·7
November ..	0·16	78·1
December ..	0·75	81·6
Yearly Total ..	46·00	
Concentration of rainfall in the period June—September ..	39·25	

TABLE II

Showing the behaviour of Co 313 gur under different conditions of storage*

Month	Sucrose %	Glucose %	Moisture %	Pore-space	Colour reading (N/4 solution)	Turbidity (N/4 solution)	Milli-equi-valents acid in 100 g.	General observations
				I. Furnace ash blanket				
April ..	80·8	5·40	5·32	25·3	80	2·20	13·5	Hard, crystalline, colour golden yellow, taste good
May ..	80·2	4·16	4·92	24·7	80	2·12	16·0	Hard, crystalline colour dull yellow, taste good
June ..	83·6	3·85	4·62	24·5	100	2·60	17·4	Hard, crystalline, colour whitish yellow, taste good
July ..	84·8	3·85	5·26	25·5	105	2·25	18·1	do
August ..	82·6	2·50	4·86	30·1	100	2·00	18·8	do
September	81·6	1·98	5·00	30·9	95	2·00	18·8	do
October ..	81·6	1·93	4·96	29·6	90	2·33	18·8	do
				II. Bhusa Blanket				
April ..	80·8	5·40	5·32	25·3	80	2·20	13·5	Hard, crystalline, colour golden yellow, taste good
May ..	80·4	5·44	5·30	21·6	80	2·21	16·7	Hard, crystalline, colour dull yellow, taste good
June ..	79·6	5·70	6·12	20·3	150	2·10	18·5	Hard, crystalline, colour reddish, taste good
July ..	80·0	5·13	7·44	18·7	175	2·10	18·8	Hard, crystalline, colour red, taste good
August ..	74·8	4·53	6·42	15·3	200	2·25	18·7	Hard, crystalline, colour red, taste good
September	72·8	4·7	6·20	14·0	200	2·20	19·4	Moist, crystalline, colour red, taste good
October ..	73·0	4·53	6·06	14·1	200	2·20	20·0	Moist, crystalline, colour red, taste good

* Data for other varieties omitted for brevity sake.

TABLE II—(*Contd.*)

Month	Sucrose %	Glucose %	Moisture %	Pore-space	Colour reading (N/4 solution)	Turbidity (N/4 solution)	Milli-equivalents acid in 100 g.	General observations
			III. Powdered charcoal blanket					
April ..	80·8	5·40	5·32	25·3	80	2·20	13·5	Hard, crystalline, colour, golden yellow, taste good
May ..	79·4	5·31	5·40	25·5	100	2·20	16·6	Hard, cryastalline, colour dull yellow, taste good
June ..	76·6	5·50	5·70	22·8	220	2·64	20·0	Some what moist and soft, crystalline, colour dark red, taste slightly deterioted
July ..	76·0	5·31	7·22	18·8	225	2·11	25·1	More soft, crystalline, colour dark red, somewhat sour
August ..	72·4	4·81	7·50	17·6	225	2·08	28·2	do
September	72·6	4·76	8·22	14·0	250	2·08	28·0	do
October ..	72·8	4·65	8·08	14·2	245	2·04	28·2	do
			IV. Exposed to atmosphere					
May ..	80·4	4·79	4·78	27·0	55	1·84	12·9	Hard, crystalline, colour, bright, golden, taste good
June ..	79·2	6·70	12·00	24·8	70	1·90	14·0	Hard, crystalline, colour dark brown, taste good
July ..	79·0	8·10	10·06	14·1	97	1·95	17·2	Soft, very moist, crystalline, taste fair, colour dark brown, some fungus
August ..	77·2	10·26	12·14	..	110	1·82	18·3	Partially liquefied taste bad, colour dark red, fungus growth
September	..	..	..	..	..	..	..	Complete decomposition, not analysed
October ..	..	..	..	..	..	..	..	do

TABLE III

Showing comparison of ash and bhusa blankets

(Year : 1945)

Varieties : Co 313 and Co 453*

(Bhindi mucilage treatment)

Storage : / Characters 1	Bhusa 2	Ash 3	S.E. ± 4	Conclusions 5
Sucrose ..	75·97	81·03	1·22	Significant
	77·34	82·17	1·02	Significant
Glucose ..	5·12	3·17	0·43	Highly significant
	5·00	3·38	0·23	Highly significant
Moisture ..	6·70	4·60	0·42	Highly significant
	6·12	4·99	0·20	Highly significant
Pore space ..	18·27	24·37	1·25	Significant
	19·90	27·23	2·34	Non-signifiant
Colour ..	207	162	6·1	Significant
	155	93	12·7	Significant
Turbidity ..	2·11	2·10	0·02	Non-significant
	2·18	2·21	0·07	Non-significant
Acidity ..	18·80	16·15	0·07	Highly significant
	17·94	17·34	0·14	Significant

* Upper figures relate to Co 453.
Lower figures relate to Co 313.
The figures are means of the whole period.

TABLE IV

*Showing comparison of ash and charcoal blankets**

(Year : 1945)

Variety : Co 313

(Sodium carbonate treatmens)

Storage : / Characters 1	Ash 2	Charcool 3	S.E. 4	Conclusion 5
Sucrose ..	82·23	75·80	1·07	Highly significant
Glucose ..	2·79	5·25	0·58	Significant
Moisture ..	4·62	6·78	0·39	Highly significant
Pore space ..	27·01	19·74	1·16	Significant
Colour ..	114	192	13.9	Highly significant
Turbidity ..	2·30	2·19	0·05	Non-significant
Acidity ..	17·86	22·80	1·16	Significant

* The figures are the means of the whole period.

TABLE V

*Showing comparison of ash, bhusa and charcoal blankets**

(Year : 1946)

Varieties : Co 313, Co 395, Co 453, Co 513

(Castor seed extract treatment)

Storage : Characters 1	Bhusa 2	Charcoal 3	Ash 4	S.E. 5	C.D. at 5% 6	C.D. at 1% 7	Conclusions 8
Sucrose ..	73·04	72·26	79·90	0·46	1·294	1·716	5%: Ash Bhusa Charcoal†
Glucose ..	6·44	6·64	4·05	0·16	0·449	0·597	1%: Ash Bhusa Charcoal 5%: Charcoal Bhusa Ash†
Moisture ..	8·15	8·28	5·03	0·22	0·619	0·821	1%: Charcoal Bhusa Ash 5%: Charcoal Bhusa Ash†
Pore space ..	19·25	18·62	25·78	0·37	1·041	1·381	1%: Charcoal Bhusa Ash 5%: Ash Bhusa Charcoal†
Colour ..	122·3	121·7	67·3	3·80	10·696	14·184	1%: Ash Bhusa Charcoal 5%: Bhusa Charcoal Ash†
Turbidity ..	1·94	1·98	2·05	0·013	0·037	0·0485	1%: Bhusa Charcoal Ash† 5%: Ash Charcoal Bhusa†
Acidity ..	18·93	20·52	13·08	0·51	1·42	0·88	1%: Ash Charcoal Bhusa† 5%: Charcoal Bhusa Ash† 1%: Charcoal Bhusa Ash

* The figures are the means of the whole period. † Highly significant.

TABLE VI

*Showing a comparison of keeping qualities of varieties**

(Year : 1946)

Varieties : Co 313, Co 395, Co 453, Co 513, B 03

(Castor seed extract treatment)

Storage methods : Ash and Bhusa

Variety : Characters 1	Co. 313 2	Co 395 3	Co 453 4	Co 513 5	B. O. 6	S.E. 7	C.D. at 5% 8	C D. at 1% 9
Sucrose ..	1·976	1·971	1·971	1·982	2·003	0·0035	0·0010	0·0013
Glucose ..	1·951	2·014	2·002	2·064	2·046	0·0191	0·051	0·068
Moisture ..	2·110	2·121	2·096	2·104	2·125	0·0254	0·0718	
Pore space ..	1·932	1·991	1·970	1·930	1·961	0·009	0·0254	0·034
Colour ..	2·093	2·088	2·087	2·088	2·078	0·0197	0·056	
Turbidity ..	2·032	2·019	1·988	2·007	1·995	0·004	0·0114	0·0153
Acidity ..	2·084	2·071	2·028	2·081	2·076	0·020	0·057	

* The periodical data have been expressed as percentages of the initial figures and then analysed after logarithmic transformation. The results relate to transformed variates.

TABLE VI—*Contd.*

			Conclusions					
			10-					
Sucrose	..	5%:	B. O. 3	Co 513	Co 453	Co 395	Co 313	Highly significant
		1%:	B. O. 3	Co 513	Co 453	Co 395	Co 313	
Glucose	..	5%:	Co 513	B. O. 3	Co 395	Co 453	Co 313	Highly significant
		1%:	Co 513	B. O. 3	Co 395	Co 453	Co 313	
Moisture	..	5%:	B. O. 3	Co 395	Co 313	Co 513	Co 453	Non-significant
Pore space	..	5%:	Co 395	Co 453	B.O. 3	Co 313	Co 513	Highly significant
		1%:	Co 395	Co 453	B.O. 3	Co 313	Co 513	
Colour	..	5%:	Co 313	Co 395	Co 513	Co 453	B. O. 3	Non-significant
Turbidity	..	5%:	Co 313	Co 395	Co 513	B. O. 3	Co 453	Highly significant
		1%:	Co 313	Co 395	Co 513	B. O. 3	Co 453	
Acidity	..	5%:	Co 313	Co 513	B. O. 3	Co 395	Co 453	Non-significant

TABLE VII

*Showing effect of juice treatment on keeping quality**

(Year : 1946)

Varieties : Co 313, Co 453, Co 513

(Bhindi mucilage and castor seed extract treatments)

Storage : Bhusa

Treatments / Characters 1		Bhindi mucilage 2	Castor seed extract 3	S.E. 4	Conclusions 5
Sucrose	..	1·947	1·955	0·003	Significant
Glucose	..	2·062	2·110	0·017	Significant
Moisture	..	2·218	2·227	0·007	Non-significant
Pore space	..	1·919	1·875	0·007	Highly significant
Colour	..	2·099	2·231	0·015	Highly significant
Turbidity	..	1·978	2·003	0·003	Highly significant
Acidity	..	2·147	2·147	0·004	Non-significant

* The periodical data have been expressed as percentages of the initial figures and then analysed after logarithmic transformation. The results relate to the transformed variates.

TABLE VIII

Showing loss in weight % under different storage blankets

Month	Percentage loss in wt.		Percentage loss in wt.	
	In ash blanket	In bhusa blanket	In ash blanket	In bhusa blanket
	Co 313 Gur		Co 453 Gur	
1	2	3	4	5
June ..	0	0	0	0
July I ..	0	0	3·8	0
July II ..	6·2	4·0	5·5	4·2
August I ..	10·0	5·0	8·3	4·0
August II ..	16·0	15·0	15·7	11·1
September I ..	16·6	15·4	19·2	22·2
September II ..	21·4	22·2	20·0	22·7
October ..	18·1	27·3	20·8	25·0

Fig. 1 demonstrates graphically the change in weight during the course of storage and Fig. 2 the variations in physical and chemical attributes during storage under different conditions.

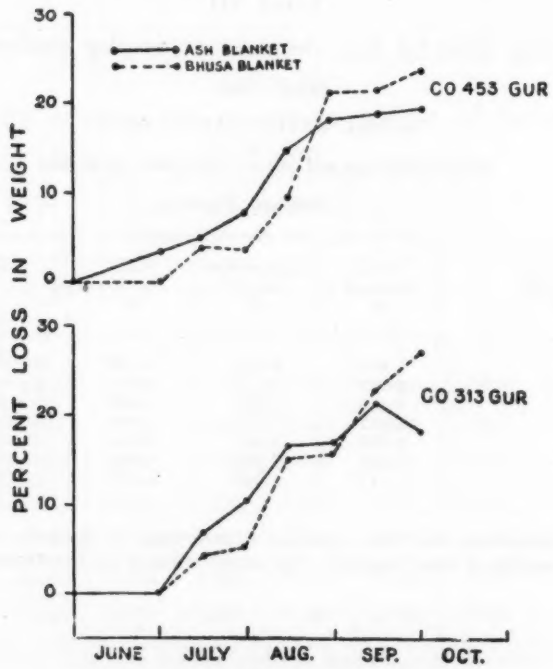


FIG. 1. Showing weight losses in storage

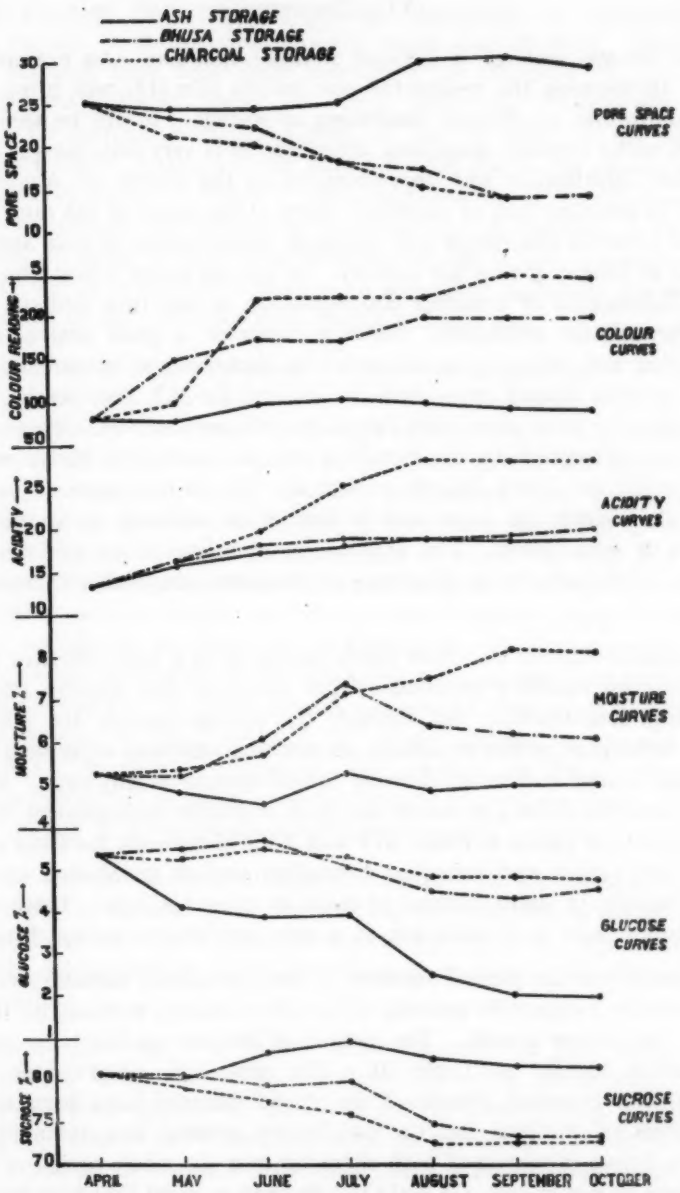


FIG. 2. Variation in properties of Co 313 Gur under different storage blankets

VI. Discussion

(i) *General features in different storage conditions.*—An examination of Table II, showing the results for one variety (Co 313) will bring out the general features in different conditions of storage. It will be seen that in storage under exposed conditions, deterioration is very fast, the gur suffering complete liquefaction and decomposition in the month of August which results in complete loss of material. Even at the onset of the rains, a good deal of moisture absorption and softening, deterioration in taste and colour, as well as fungus growth are noticed. In storage under wheat bhusa, there is no liquefaction or complete decomposition at any time and the product lasts through the monsoons. There is, however, a good deal of moisture absorption and softening accompanied by darkening in colour and in case of all varieties studied other than Co 313 and Co 513, considerable damage with respect to taste along with fungus growth are observed. These changes are clearly perceptible by the month of July and storage in bhusa must also be regarded as a very imperfect method. The performance of powdered charcoal is much the same and in respect of tendency to sourness, this blanket is even worse. The expectation regarding better performance on account of the adsorptive properties of powdered charcoal is therefore, not fulfilled.

Furnace ash, on the other hand, proves to be a very effective blanket, providing an excellent protection from moisture and thereby completely inhibiting deterioration. All through the storage period, the gur stored in ash remains in perfect condition, maintaining hardness, crystalline texture, taste and colour and never showing fungus growth at any time. In many cases where the initial gur colour was dark, a distinct improvement in colour was noticed. A glance at Plates XIV and XV will show the hard and compact form, light colour and unbroken crystalline surface maintained by an ash stored sample, in sharp contrast to those in other blankets. These remarkable results point to furnace ash as a very satisfactory storage blanket.

Apart from the general features, it was considered necessary to obtain a statistically comparable estimate of the three storage methods on the basis of the quantitative criteria. The method of furnace ash has been compared with bhusa blanket in Table III. The periodical observations on the physical and chemical criteria of the stored material have been subjected to analysis of variance and the conclusions entered in column (5). The averages drawn in respect of each character over the whole period of storage have been shown in cols. (2) and (3). Bearing in mind that high values are desirable for sucrose and pore space, and low values for the remaining

criteria, it is revealed that ash storage has maintained its superiority over bhusa storage over the whole period under consideration. Similar analyses of variance have been done comparing the efficiency of furnace ash with charcoal blanket. Table IV would show that furnace ash is superior also to charcoal blanket. Tables III and IV refer to the data collected during 1945. Analysis of the data collected during the subsequent year 1946 has been shown in Table V which supports the above conclusion in favour of the furnace ash except for one character (turbidity). A basis of comparison of bhusa storage with charcoal storage is also available in this table. It will be noticed that there is no significant difference between the two methods so far as the keeping quality is concerned.

The superiority of the ash blanket being clearly established it now remains to be examined how the product stored in it undergoes losses in weight, as compared to the country method of bhusa storage. Experiments were performed with respect to a large number of samples, in different years in which blocks of gur were stored in ash and bhusa and their percentage losses in weight periodically recorded during the storage period from June to October, two typical examples being depicted in Table VIII and Fig. 1. It will be seen that although in the initial stages, samples in ash storage tend to lose somewhat more in weight, the loss becomes distinctly greater for the bhusa stored samples by the beginning of September. Further experiments on a semi-large scale conducted in two successive years, in which the material was left undisturbed for the whole storage period and weighed in October, showed net losses of 17.2 and 11.7% in ash storage, as opposed to 32.0 and 34.3% in bhusa. Moreover, the product in bhusa storage is moist while that stored in ash is perfectly dry and in good condition. The results, thus, speak in favour of the ash blanket from this standpoint as well.

On taking out gur from ash storage, the removal of ash adhering to it presents no difficulty on account of the perfectly dry condition of the blocks and dusting with a piece of cloth is enough to get rid of it. The storage material being a bye-product of gur manufacture, no extra cost is incurred on this account.

(ii) *Varietal differences.*—In Table VI an attempt has been made to study the varietal effects on keeping quality. In order to estimate the differences in keeping quality, the values of the different criteria obtained during different months have been expressed as percentages of the initial figures and analyses of variance have been done after logarithmic transformation. The results furnished in Table VI relate to the transformed variates. It will be seen that a combined assessment of the keeping quality

in respect of the different characters does not lead to any definite conclusion in favour of any variety.

So far as the general features are concerned, Co 313 and Co 513 appear to be definitely superior in that they do not acquire bad taste or suffer from fungus growth (unlike other varieties) at any time during storage by the common bhusa method.

(iii) *Treatment effect.*—Table VII sets out the results of the analyses of variance in respect of two treatments, bhindi mucilage and castor seed extract. The method of analysis relevant to this table is the same as in the case of varietal differences reported above. Since there are only two methods, the conclusion recorded in col. (5) would indicate the superiority or otherwise of a particular treatment over the other. It will be observed that in respect of sucrose, the castor seed extract treatment shows smaller deterioration, moisture and acidity differences are non-significant while the other characters indicate the superiority of the bhindi mucilage treatment. In view of these conflicting trends, no definite conclusion can be drawn as to the relative keeping qualities associated with the two treatments.

(iv) *Changes in physical and chemical properties under different conditions of storage.*—The observations with respect to changes in the various physical and chemical properties under different conditions of storage are briefly discussed here. A graphical representation for one typical sample will be found in Fig. 2.

(1) *Sucrose.*—Under exposed conditions, sucrose content falls gradually in course of storage and by the month of August, losses of 3 to 6 per cent. are observed after which there is complete loss of material, when analysis cannot be performed. In bhusa storage, likewise, the deterioration with respect to sucrose by the beginning of August is between 3 to 7% and this continues steadily upto the end of the storage period, losses of 6 to 10% being observed in October. The behaviour of the charcoal blanket is very similar to bhusa, the ultimate loss in sucrose amounting to about 8%. In striking contrast to these, the ash blanket does not permit of any loss in sucrose but an overall gain of 1 to 2% is observed and in some cases, sucrose maxima are exhibited in the months of June and July. We may, therefore, characterise the trend in sucrose changes with respect to gur samples in different stages of deterioration as follows:—

Good condition	..	No loss of sucrose during storage.
Average condition	..	Progressive loss in sucrose amounting ultimately, upto 10 per cent.

Bad condition	..	Loss in sucrose upto 6 per cent. early in August followed by total loss of material.

(2) *Glucose.*—Samples exposed to the atmosphere show steady increases in glucose content, amounting to values about 100% higher than original early in August after which there is complete decomposition. In the bhusa and charcoal blankets, this rise is very effectively checked and glucose content does not materially alter in storage. The contrary behaviour is noticed in ash storage when glucose falls steadily, ultimately reaching values of the order of 30–40% of the original. Glucose changes may be thus characterised as under:

Good condition	..	Gradual fall in glucose to values 30–40% of the original.
Average condition	..	Glucose does not materially alter.
Bad condition	..	Rises of the order of 100% occur by August, after which complete decomposition is observed.

(3) *Moisture.*—Exposed samples double their moisture contents by August before the complete liquefaction stage is reached. In bhusa, there is an average overall increase of about 2% from May to October but it is not uncommon to find maxima during the period July to September. Under charcoal blanket, however, these maxima are absent and the total rise in moisture at the end of storage amounts to about 3 per cent. The ash blanket, on the contrary, does not permit of any rise in moisture content at any time during storage but there is a slow decrease. We may thus generalise as follows:

Good condition	..	No increase in moisture content takes place but there is a reverse tendency.
Average condition	..	Net rises of 2 to 3% are observed with occasional maxima during the wettest months.
Bad condition	..	The moisture content doubles itself by the beginning of August which is followed by complete liquefaction.

(4) *Acidity.*—This property develops slowly and in exposed conditions, increases of the order of 50% are generally observed early in August before the samples liquefy altogether. The bhusa blanket is very similar, showing the same order of increase in August, the total rise being generally in the vicinity of 60%. In charcoal storage, steeper rises are observed, amounting to over 100% at the end of storage. In furnace ash, the rise is generally

within 30%. With respect to this property, the different conditions of deterioration may be characterised as under:

Good condition	..	Small increases not exceeding 30% of the original value are observed at the end of storage.
Average condition	..	Increases of the order of 50% by August, ending in total rises between 60–100% in October.
Bad condition	..	Rises of the order of 50% by August, followed by complete deterioration.

(5) *Intensity of colour.*—This increases rapidly in exposed storage conditions, doubling itself by August before complete deterioration occurs. In bhusa, darkening is slower and the total increase is generally of the order of 20–30%. Charcoal is somewhat worse than bhusa in this respect. In furnace ash, the increase in intensity of colour is generally very small and in those cases where the initial colour was high, decreases were observed on storage. The generalisation with respect to this property would therefore be:

Good condition	..	Intensity of colour increases little, and falls in many cases.
Average condition	..	A gradual and steady increase occurs.
Bad condition	..	Rapid increase takes place amounting to 100% in August before complete decomposition.

(6) *Concentration of suspended impurities.*—The turbidity of solutions shows the same behaviour under different conditions of storage in that it always maintains the same order of magnitude, thus showing that storage has no effect on the suspended impurities in gur.

(7) *Pore space.*—Pore space drops very fast under exposed conditions and is reduced to half the original value early in August even before the complete liquefaction stage is reached. In bhusa storage, the fall is much lower, 20–30% decrease being observed in the corresponding period and ultimate fall of 30–45% being recorded in October. In charcoal blanket, the trend closely follows bhusa. Under ash storage, however, the contrary behaviour is observed wherein pore space steadily increases, ultimately reaching in the month of October values 20–30% above the original. This denotes an increasingly open texture, constituting improvement in quality as compared to the gradual decrease brought about by moisture absorption and consequent collapse of the capillary spaces in all other conditions of storage. The following characteristics are therefore formulated:

Good condition	..	Tendency of pore space to increase (even by 20–30%) imparting more open texture.
Average condition	..	Slow fall in pore space, values acquired in August being 20–30% lower and in October 30–45% lower than original.
Bad condition	..	Rapid fall in pore space amounting to 50% original value by August before complete liquefaction occurs.

The changes in respect of important physical and chemical attributes of gur under storage are thus clearly brought out and characteristics associated with different conditions of deterioration elucidated.

VII. Summary

1. Under the highly humid conditions prevailing in Eastern India during the monsoon, storage of gur at this time of the year presents a serious problem as the material is apt to suffer greatly in quality as well as quantity on account of moisture absorption, resulting in inversion and fermentation which gives rise to great deterioration and loss. The country methods of storage in vogue (*e.g.*, inside a blanket of bhusa or wheat straw) gives far from satisfactory results and in these days of acute food shortage the need for evolving an effective storage method must be regarded as a national one. In these investigations, furnace ash and charcoal powder (selected respectively on account of their hygroscopic and adsorptive properties) have been examined with respect to their efficacy as storage blankets against the commonly used storage material, wheat bhusa as also storage under exposed conditions.

2. The experiments bring out the unique performance of furnace ash (from gur furnace) in affording perfect protection from moisture and saving gur under storage from the least deterioration during the rains. The reason for this behaviour obviously lies in the highly hygroscopic nature of ash, by virtue of which property, the material arrests the atmospheric moisture in itself, thus preventing it from reaching the gur stored inside. The utility of furnace ash as storage blanket has been discussed from different standpoints.

3. Experiments on changes in important physical and chemical attributes of gur under different conditions of storage reveal interesting differences in behaviour which lead to definite characteristics in respect of these properties, associated with different stages of deterioration in storage. These are stated here in brief.

(*a*) Gur stored by a satisfactory method (furnace ash) which is considered to be in good condition shows the following features: No loss in sucrose; gradual fall in glucose culminating in values of the order of 30–40% of the original; no increase in moisture, but a reverse tendency; increase in acidity not exceeding 30% at the end of the storage period; no substantial increase in colour intensity and decrease in many cases; no appreciable variation in concentration of suspended impurities; tendency of pore spaces to increase even by 20–30% denoting increasing openness in texture.

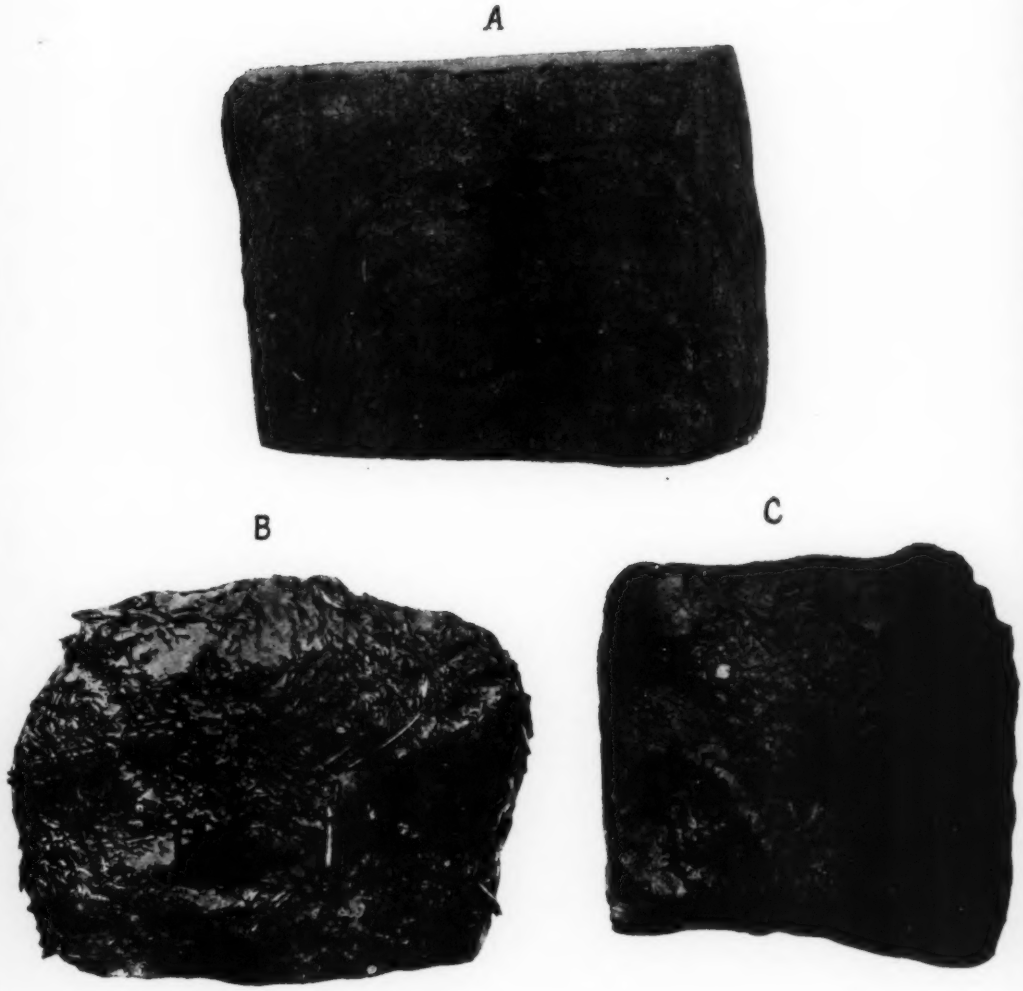
(*b*) The product stored in a blanket of ordinary efficacy (bhusa or charcoal powder) which maintains an average condition in storage has the following characteristics; progressive loss in sucrose amounting ultimately to 10%; no material alteration in glucose content; net raises of 2 to 3% in moisture with occasional maxima in the wet months; increases in acidity of the order of 50% by August ending in overall rises between 60–100% in October; steady increase in colour intensity; concentration of suspended impurities maintaining the same level; slow decreases in pore space, values acquired in August being 20–30% and in October 30–45% lower than the original, indicating gradual transition to a more closed texture.

(*c*) Gur stored in an unsatisfactory manner (exposed to atmosphere) suffers complete liquefaction by the latter half of August when it is entirely lost. Based on observations extending upto the beginning of August, the characteristics associated with gur in bad condition may be stated as under:

Loss in sucrose upto 6% and rises in glucose and moisture contents of the order of 100% early in August, increase in acidity upto 50% of the original and rapid increase in colour intensity amounting to 100% before the complete decomposition stage is reached; no variation in concentration of suspended impurities; rapid fall in pore space amounting to 50% of the original values before complete liquefaction takes place.

VIII. Acknowledgement

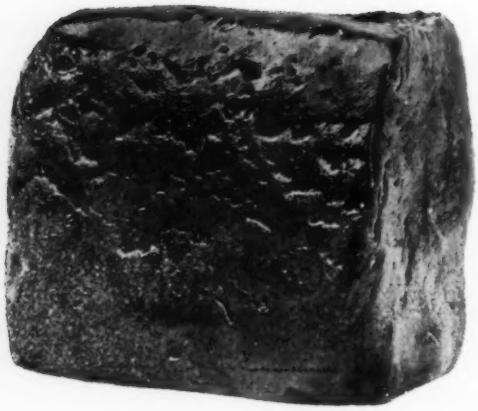
The work was carried out as part of the Sugarcane Research Scheme in Bihar financed jointly by the Bihar Government and the Indian Central Sugarcane Committee, to whom grateful thanks are due. Mr. Thakur Parsad, M.Sc., who is at present working as District Agricultural Officer, was associated with the early stages of this investigation as gur chemist. The assistance afforded by him in the preliminary trial, and that by Mr. K. S. Bandyopadhyay, Statistician, in analysing the data pertaining to different experiments reported in this paper is also acknowledged.



Shows blocks of gur preserved in different blankets. *A* denotes a block stored through the monsoon inside Furance ash; *B* in wheat bhusa and *C* in charcoal powder. It will be seen that *A* has maintained its shape, compactness and crystalline texture very well; *B* has become soft and plastic and deformed due to pressure in storage; its surface is covered all over with pieces of bhusa sticking to it; *C* has also become soft and moist and undergone deterioration in texture and wearing off at the periphery.

A

B



C

Shows the same blocks of gur as in Plate XIV, after they have been cut into similar shapes and their inner surfaces exposed, so as to demonstrate more clearly internal differences in texture. Block *A* presents a hard, compact form, light colour and an unbroken crystalline surface. On the other hand, Block *B* clearly appears to be a very dark coloured, soft and plastic solid which has hardly maintained its form even after being cut into a regular shape, its surface presenting a sticky amorphous texture, interspersed by numerous cracks and fissures. In the same way, Block *C* is dark coloured and although more compact than Block *B*, it shows a surface full of crevices and of a distinctly amorphous texture.

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THE LARVAL STAGES OF *PERICLIMENES (PERICLIMENES) INDICUS* KEMP*

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Introduction

Larvæ of several species of *Periclimenes* have been described before; but all of them belong to the subgenus *Ancylocaris*. *Periclimenes indicus* Kemp comes under the subgenus *Periclimenes* (Kemp, 1922), and an account of its larvæ should not be without interest, as it would serve to bring out the differences among the larvæ of a genus whose species are so numerous and so diverse in habits. This species was originally described by Kemp (1915) as *Urocaris indica*. Subsequently in his account of the Indian Pontoniinæ (1922) the genera *Urocaris* and *Ancylocaris* were merged in the genus *Periclimenes* as it became evident to him that the two former should be regarded as only synonyms of the latter. Evidence derived from larval structure may now be examined to ascertain whether it lends support to this arrangement.

Berried females together with larvæ and immature forms were obtained from the plankton of Adyar backwater in May and the following 3 months. Panikkar and Aiyyar (1939) have recorded the occurrence of egg-bearing females in the same locality in April, September and October also. In view of Kemp's records (1915) from Chilka lake and Ennur they remark that " it is not possible to say whether there is any well-marked breeding season for the species".

Larvæ belonging to the first stage were obtained by keeping berried females (carrying eyed eggs) in aquaria. A few of these first stage larvæ moulted in the course of 2 or 3 days, but it was found extremely difficult to get the later stages in the same manner. They were, however, present in townet collections so that a complete series was available for study.

Egg

Kemp has described the eggs as sage green in colour. Those with fully developed eyed embryos (Fig. 1) are somewhat oval in shape and measure

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0·5 mm. in length and about 0·35 mm. in breadth. The embryonic telson is armed with 6 pairs of spines on its posterior margin.

Larvæ

Stage I (Fig. 2).—Total length about 1·5 mm. The body, as is usual in the larvæ of *Periclimenes* and related genera, has a conspicuous bent at the third abdominal segment. As in the first stage of all Caridean larvæ the eyes are fused with the carapace and a minute rostrum projects forwards between them. The carapace has no other spines on any other part. The first 3 pairs of maxillipeds are well developed and functional, with exopodites tipped with large plumose setæ.

The larvæ are quite transparent, with numerous maroon chromatophores, some of which are large and branching and have yellow margins. They show the following arrangement:—

1. A pair on the dorsal side just behind the eyes. They are united together.
2. A pair at about the middle of the cephalothorax, one on each side.
3. A somewhat smaller pair on the second abdominal segment, lateral in position.
4. A pair at the middle of the third abdominal segment also placed laterally.
5. A pair at the base of the telson.
6. A pair on the ventral side between the base of the antennæ, into which their branches enter.

Numerous dot-like pigment bodies are also present scattered at the base and endopodites of the maxillipeds, tips of the antennular peduncles and extremity of the telson.

Antennule (Fig. 3).—It consists of a peduncle and the outer flagellum. The latter is unsegmented and carries 4 terminal æsthetes and a short plumose seta. Inner flagellum is represented by a plumsore seta.

Antenna (Fig. 4).—It has an unjointed peduncle, flagellum and scale. Flagellum shows no segmentation and is tipped with a small spine and a seta. The tip of the scale is distinctly jonted, 4 joints being distinguishable. Along its inner margin and tip there are 9 long plumose setæ and a short one on the outer margin at the level of the first joint. Anterior to this and external to the outermost terminal seta there is a short hair-like one. Proximally behind the first seta there is a small papilla.

Mandible (Fig. 5).—Between the incisor and molar portions there is a prominent serrated tooth.

Maxilla I (Fig. 6).—It has two endites and a palp. Both the endites have each 5 short setæ, those of the distal being larger. Palp is short, unsegmented and terminated by a small seta.

Maxilla II (Fig. 7).—Only 3 endites are present, the proximal being larger than the other two. First and third have 2 setæ each and the second 1. Palp projects slightly beyond the anterior extremity of the scale, and has a single terminal seta. Scale is fringed with 5-6 plumose setæ, one of which, arising from the posterior extremity is the largest.

Maxilliped I (Fig. 8).—Protopodite is armed with 2 setæ distally. Endopodite is short, unjointed and bears 3 unequal setæ terminally. Exopodite shows a number of constrictions, dividing it indistinctly into 8 or segments. It has 4 plumose swimming setæ at the tip and a hair-like pair at the distal joint.

Maxilliped II (Fig. 9).—Protopodite and exopodite are similar to those of the first. Endopodite has 3 segments, second and third being armed with 2 and 5 setæ respectively.

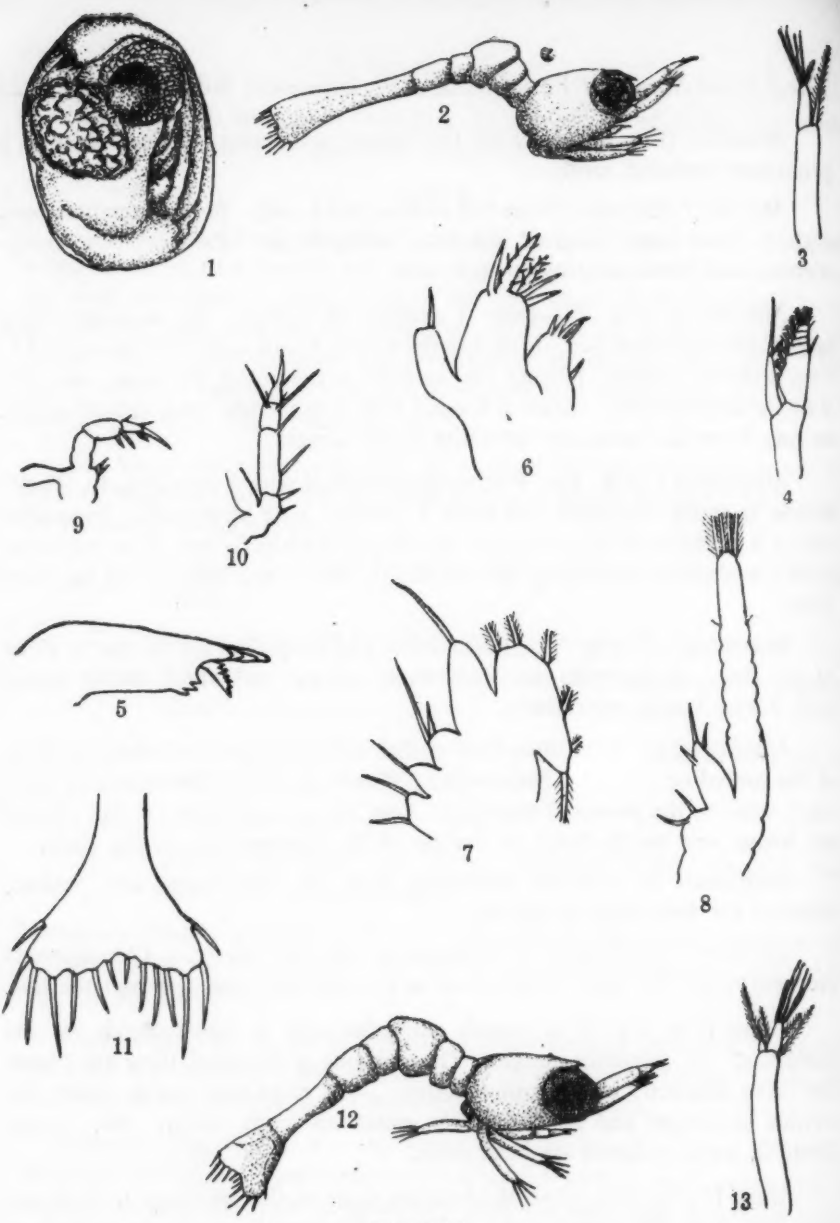
Maxilliped III (Fig. 10).—Protopodite and exopodite are similar to those of the preceding 2 pairs. Endopodite differs from that of the second in having 2 setæ on the proximal segment. Also the pair of setæ of the second are longer and nearly reach to the tip of the terminal seta of the third.

Rudiments of 3 of the remaining pairs of peræopods are present, those of the first being biramous.

Abdomen.—It consists of 5 segments and the telson. The segments are unarmed, the third being much larger than the others, except the last.

Telson (Fig. 11).—It is broadly triengular with a small notch in the middle of its posterior margin. On each side of the notch there are 7 setæ, one being distinctly on the outer margin. The third and fourth from the outside are longer and approximately equal to each other. All, except 1 and 2, are on ciliated on both sides.

Stage II (Fig. 12).—Length about the same as that of Stage I. Eyes are now free from the carapace and stalked. Rostrum is much longer than in Stage I, though it does not reach to the extremity of the eyes. At its base a small knob or papilla has developed. Anterolateral angles of the carapace are produced into very small pterygostomian spines. Peræopods 1 and 2 have become functional.



FIGS. 1–13

Fig. 1. Fully developed egg. ×75.
Fig. 2. Stage I: Entire animal. ×36.
Fig. 3. Do. Antennule. ×75.
Fig. 4. Do. Antenna. ×75.
Fig. 5. Do. Mandible. ×330.
Fig. 6. Do. Maxilla I. ×330.
Fig. 7. Do. Maxilla II. ×330.
Fig. 8. Stage I: Maxilliped I. ×198.
Fig. 9. Do. Maxilliped II. ×75.
Fig. 10. Do. Maxilliped III. ×75.
Fig. 11. Do. Telson. ×75.
Fig. 12. Stage II: Entire animal. ×36.
Fig. 13. Do. Antennule. ×75.

Antennule (Fig. 13).—It shows little difference from that of Stage I. Outer flagellum seems to have 5 æsthetes and the distal end of the peduncle carries 4 to 5 setæ. Inner flagellum is in the form of a tiny papilla with a seta at its tip.

Antenna.—It shows hardly any change.

Mandible.—There may be 2 serrated teeth between the incisor and molar processes.

Maxilla I.—Distal endite has a couple more of setæ.

Maxilla II (Fig. 14).—Palp has two unequal terminal setæ. Scale has 7 plumose setæ, 5 of which are anterior in position and 2 posterior.

Maxillipeds I and II.—They are practically as they were in Stage I.

Maxilliped III (Fig. 15).—Protopodite and exopodite are as in Stage I. Endopodite has 4 segments armed with 2, 0, 2 and 5 setæ repectively.

Legs I and II (Fig 16).—Protopodite is armed with 2 setæ as in maxillipeds. Exopodite has 4 swimming setæ. Endopodite has 4 segments carrying 2, 1, 2 and 3 setæ respectively, that of the second being on the outer side.

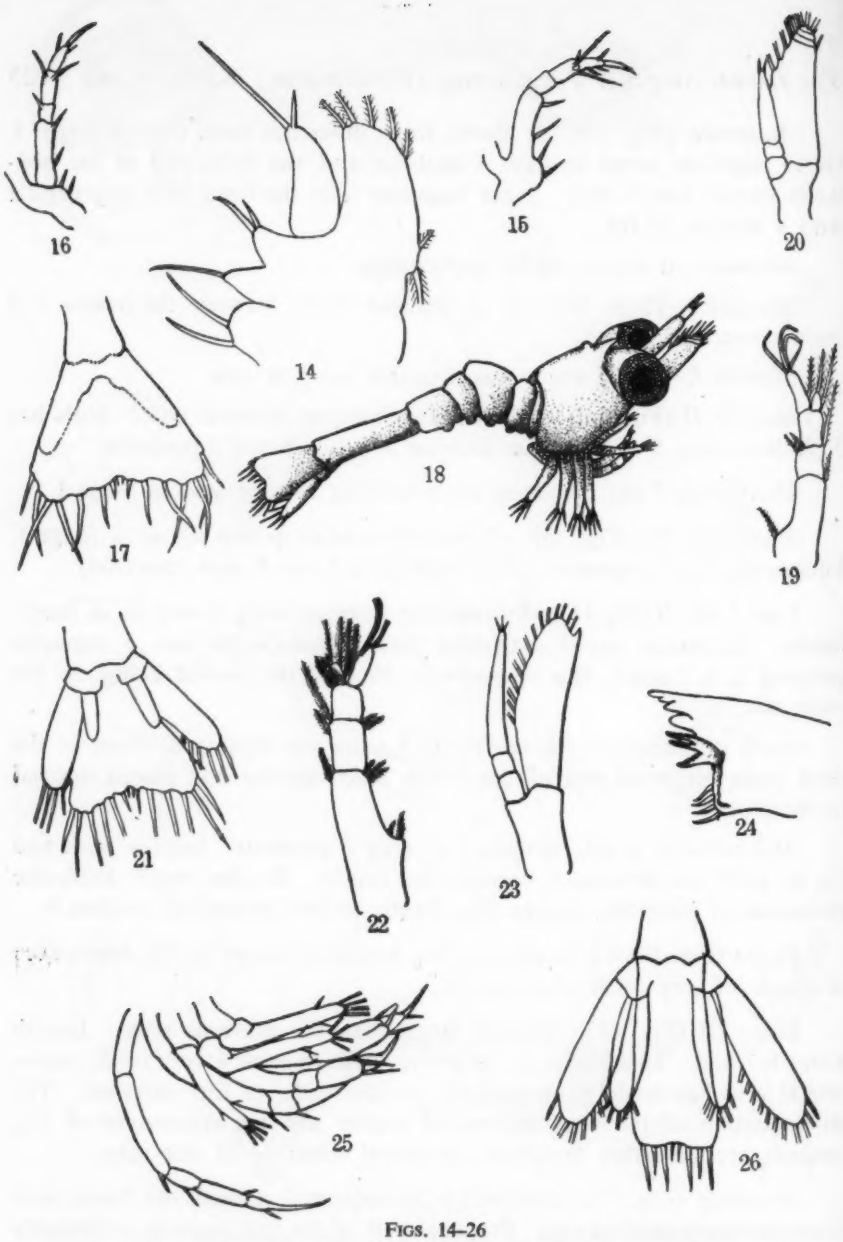
Small rudiments of the remaining 3 pairs are developed, those of the third being biramous and of the fourth much smaller and placed internal to the others.

Abdomen.—It is still composed of only 5 segments; but the sixth and the uropods are developed beneath the cuticle. So also small knob-like rudiments of pleopods, though they hardly project beyond the surface.

Telson (Fig. 17).—It is more or less unaltered except for the appearance of a pair of very small inner spines.

Stage III (Fig. 18).—Slightly larger than the previous stage. Length about 1·7 mm. In addition to the pterygostomian spine a very small supraorbital also has made its appearance on each side of the carapace. The differentiation of the sixth abdominal somite and the appearance of free uropods are the other important structural advances of this stage.

Antennule (Fig. 19).—Peduncle is three-jointed, though the basal joint is not distinctly marked out. Proximal part of the first segment is distinctly swollen, and carries a seta. Large plumose setæ are borne at the tip of the third joint and a few on the inner margin of all the joints. A pair of quite small setæ are present at the junction of the second and third segments on the outer side. Both flagella are slightly longer, but are otherwise unaltered.



FIGS. 14–26

Fig. 14. Stage II: Maxilla II. ×330.
Fig. 15. Do. Maxilliped III. ×75.
Fig. 16. Do. Leg I. ×75.
Fig. 17. Do. Telson. ×75.
Fig. 18. Stage III: Entire animal. ×36.
Fig. 19. Do. Antennule. ×75.
Fig. 20. Do. Antenna. ×75.
Fig. 21. Stage III: Telson and Uropods. ×75.
Fig. 22. Stage IV: Antennule. ×75.
Fig. 23. Do. Antenna. ×75.
Fig. 24. Do. Mandible. ×330.
Fig. 25. Do. Peræopods. ×75.
Fig. 26. Do. Telson and Uropods. ×75.

Antenna (Fig. 20).—Two segments are distinctly marked out at the base of the flagellum. Excluding the small hair the scale has 12 setæ along inner margin and tip.

Mandible and the 2 pairs of maxillæ are little altered.

The 3 pairs of maxillipeds also exhibit hardly any change. So too legs 1 and 2.

Rudiments of peræopods 3, 4 and 5 are larger, that of the fourth being still smaller and in the same position.

Abdomen.—The sixth segment has been added. Pleopods are still in the form of round knobs.

Uropods (Fig. 21).—They are developed and functional, each having protopodite, a short unarmed endopodite and a much larger exopodite provided with 6 setæ along inner margin and tip. Telson is unchanged.

Stage IV.—It is slightly less than 2 mm. in length. The tubercle at the base of the rostrum noticed in the 2 previous stages is conspicuous now and anterior to it on the rostrum itself there is a tooth. In addition to the supra-orbital and pterygostomian spines there is an equally small spine which, according to Gurney, is the branchiostegal. All peræopods are now well developed and jointed except the fourth, the last 2 pairs having no exopodites. Pleopods are in the form of uniramous papillæ.

Antennule (Fig. 22).—Both on the outer and inner sides of the peduncle there are numerous plumose setæ. Inner flagellum is still unsegmented; outer seems to be two-jointed, the proximal joint having 3 æsthetes and distal 4, one of which is setiform.

Antenna (Fig. 23).—Flagellum is almost as long as scale, but still shows only the 2 basal joints. At the tip there may be 2 or 3 setæ. A short spine has developed at the extremity of the outer margin of the scale, which has lost all trace of segmentation, its tip and inner margin carrying 14 setæ.

Mandible (Fig. 24).—Three serrated teeth are present between the incisor and molar processes.

Maxillæ and Maxillipeds have not changed much.

Peræopods (Fig. 25).—First 3 pairs are biramous with functional exopods similar to those of the maxillipeds. Endopodites are four-jointed, the first and second being chelate. The fourth and fifth are directed forwards, the fifth lying along the midventral line. All the joints carry setæ. The fourth is still rudimentary, not even half as long as the fifth and bears no setæ.

Uropods (Fig. 26).—Endopodites are also setose in this stage, their tips carrying 5 plumose setæ. Exopodites have 11 setæ along inner margin and tip, the outermost at the tip being spine-like.

Telson.—It has undergone considerable alteration in shape, being now almost rectangular with the sides parallel. The posterior border is straignt and carries 4+4 spines, the innermost being quite small. A small tooth is present on the outer margin at about a fourth of its length from the posterior end.

Stage V.—The length slightly exceeds that of Stage IV. This is the last larval stage and the most important advance which it shows over the previous is in respect of the pleopods, which have grown considerably, and consist of a peduncle, a small endopod and much larger exopod, both devoid of setæ.

Antennule.—The enlargement at the base of the first segment of peduncle has grown and projects as a prominent triangular process with a few short setæ at its base and angle. Inner flagellum has grown slightly and is as long as the first segment of the outer flagellum.

Antenna.—Scale has now 15 plumose setæ besides the spines at the extremity of the outer margin. Flagellum is distinctly longer than scale and besides the 2 segments at its base, has 1 at the extremity also. Peduncle is distinctly two-jointed with a minute spine on the inner side of the extremity of the distal segment.

Mandibles, maxillæ and maxillipeds are as they were in the previous stage.

Peræopods.—They are better developed and the endopodites show 5 segments. The fourth leg is now fully developed and jointed. It is only slightly shorter than the fifth. The setæ of the penultimate segment are not larger than those of the other legs.

Uropods.—Endopodite has 9 setæ along inner margin and tip, besides 2-3 hair-like ones arising, not from the edge, but from the inner surface close to it. Exopodite is fringed with 14 setæ.

Telson (Fig. 26).—All the spines of fourth stage are present in this stage also. On the sides, however, 2 very small spines have appeared so that there are 3 pairs of lateral spines, the most proximal pair of which are identical with those of Stage IV.

Post-Larva

Length is about 2·5 mm. The rostrum (Fig. 29) extends forwards to the middle of the eyes and has the crest dorsally, so prominent in the adult.

It carries 2 teeth in addition to the basal papilla. The pterygostomian, branchiostegal and supraorbital spines of the larvæ have disappeared, and an antennal spine, and behind it a small hepatic spine have developed. The legs are all well developed and the dactyli of the last 3 pairs have the characteristic spine (Fig. 37) producing the biunguiculate appearance peculiar to the subgenus *Periclimenes*. These and other characters make their identification in plankton material comparatively easy.

Antennule (Fig. 30).—Inner flagellum has 3 and outer 4 segments. Second segment of the outer flagellum has a process carrying 3-4 æsthetes, which later develop into one of the 2 branches. The outer triangular process of the basal segment is more prominent and the outer margin anterior to it is convex, but does not project forwards beyond the insertion of the second segment as in the adult animal. The two distal segments of the peduncle together measure about half of the first.

Antenna (Fig. 31).—Spine on outer margin of scale reaches almost to the tip. Flagellum is more than half the body in length.

Mandible (Fig. 32).—Incisor and molar processes are widely separated, the former having 3-4 teeth. Serrated teeth of the larvæ have disappeared and there is no palp.

Maxillæ I and II do not show any change.

Maxilliped I (Fig. 33).—Endopodite is quite small. Masticatory processes of the protopodite are well developed, and closely beset with setæ. A small knob-like epipodite is present.

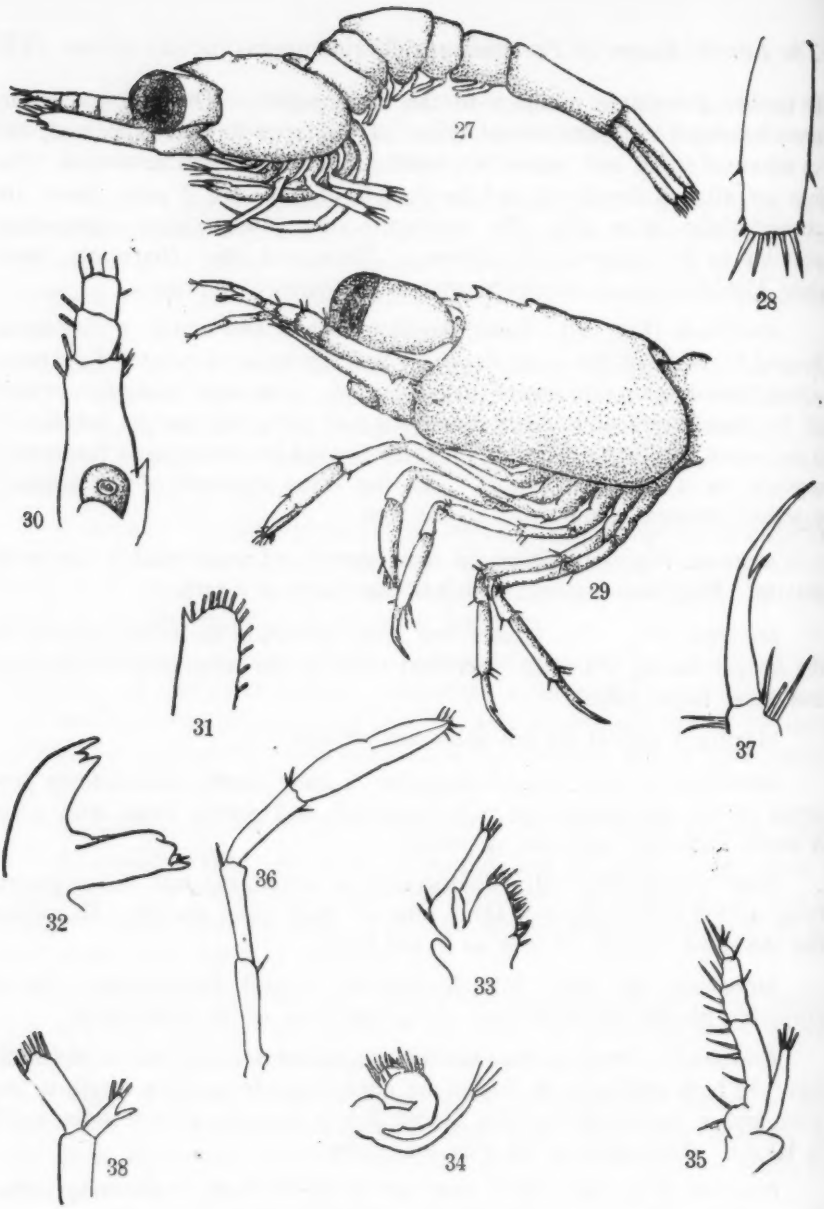
Maxilliped II (Fig. 34).—Endopodite is curved, the last two segments being armed with numerous short setæ on their outer margin. Exopodite has the same number of setæ as in the larvæ.

Maxilliped III (Fig. 35).—Endopodite is still four-joinetd. Tip of exopodite reaches the extremity of the first joint of the endopodite.

Peræopods.—Proportionate size of the segments is not that of the adult legs. In both chelipeds the fingers are approximately equal in length to the palm and in the second leg (Fig. 36) the first 3 segments do not differ much in length. Rudiments of all gills are visible.

Pleopods (Fig. 38).—Both rami are provided with swimming setæ. A small appendix interna is carried by each endopodite. Uropods are longer than the telson.

Telson.—The posterior spines of the telson of the only available specimen had been broken off. But in a slightly longer specimen there are 2 pairs



FIGS. 27–38

Fig. 27. Stage V: Entire animal. ×36.
Fig. 28. Do. Telson. ×75.
Fig. 29. Post-larva: Anterior part of body. ×45.
Fig. 30. Do. Antennule. ×75.
Fig. 31. Do. Antennal scale. ×75.
Fig. 32. Do. Mandible. ×198.
Fig. 33. Post-larva: Maxilliped I. ×75.
Fig. 34. Do. Maxilliped II. ×75.
Fig. 35. Do. Maxilliped III. ×75.
Fig. 36. Do. Second leg. ×75.
Fig. 37. Do. Tip of leg 5. ×198.
Fig. 38. Do. Pleopod. ×75.

of lateral spinules and 3 pairs of posterior spines, the second of which are larger than the others.

Discussion

Our knowledge of the larvæ belonging to the sub-genus *Ancylocaris* is fairly extensive. Gurney has described the complete series of stages of *P. calmani* (1927) and *P. americanus* (1936) and also the first stage of 3 other species, namely, *P. grandis*, *P. agag*, and *P. diversipes* (1938). In addition to these the first stage of *P. brevicarpalis* has been described by Nair (1947). Excluding *P. diversipes* and *P. brevicarpalis* larvæ of other species agree very closely and a summary of the characters of the subgenus based on their characters has been given by him (1938). Regarding *P. diversipes* he has remarked that it differs so much from the others " that one would suppose it should be referred to a distinct genus or subgenus. Such differences are the very well-developed antennal papilla, form of endopod of maxillule, presence of 5 setæ on exopod and absence of basal lobe on endopod of maxilla and terminal spines of maxilliped III." In 3 of these characters, namely presence of antennal papilla, absence of a basal lobe on enopod of maxilla and terminal spines of maxilliped III *P. brevicarpalis* agrees with *P. diversipes*. *P. indicus* agrees with *P. diversipes* in all these characters and thus differs from most of the species of the *Ancylocaris* group. The other more important differences are given below stage by stage.

Stage I.—Antennal scale has only one outer seta; endites of maxilla have much fewer setæ (the number is the same as in *P. brevicarpalis*); exopodites of maxillipeds constricted so as to produce the appearance of segmentation and provided with at least one hair-like pair behind the terminal setæ.

Stage II.—Rudiment of leg 5 does not show any marked difference in size.

Stage III.—Fifth legs are not long and styliform, but only rudiments, hardly larger than those of legs 3.

Stages IV & V.—A branchiostegal spine is present; flagellum of antenna has two basal segments and in Stage V a third segment at the tip so that it is four-jointed; leg 4 is an unarmed rudiment in Stage IV, not reaching to the extremity of the first segment of leg 3; in Stage V the setæ of the last 2 segments are not mush different from those of leg 3; 2 extra pairs of minute lateral spines on the telson in Stage V.

Besides the species mentioned above, the first larvæ of the following species belonging to other genesa have also been described:—

1. *Pontonia phallusiæ* (Gourret, 1884)
2. *Pontonia tyrrhena* (Caroli 1926)
3. *Typton spongicola* (Lebour, 1926; Caroli, 1926)
4. *Harpilius beaupresi* (Gurney, 1938)
5. *Harpilius gerlachei* (Gurney, 1938)
6. *Coralliocaris graminea* (Gurney, 1938)

Lebour has remarked that in its segmented antennal scales, the form of its antennules and general build the larva of *Pontonia* resembles that of *Typton*. It may be of interest to note here that the exopodites of the maxillipeds in the latter show indications of 3 segments, agreeing in this respect with the present species, though here the number of segments is more. In regard to the other species Gurney has pointed out that *Harpilius beaupresi* and *Coralliocaris graminea* resemble very closely *P. diversipes*, while *H. gerlachei* agrees with the other species of *Periclimenes*. He thinks therefore that their larval structure indicates quite a different generic grouping of the adults from that which is adopted by Kemp.

Reference has already been made to the similarity between *P. indicus* and *P. diversipes* in those characters in which the latter differs from other *Periclimenes* species. It is therefore hardly necessary to indicate how the former agrees with *H. beaupresi* and *C. graminea.*

The mode of development of the fourth and fifth legs and the arrangement of the setæ on the last 2 segments, especially of leg 4, have to be considered in this context. The retarded development of leg 4 and the precocious development of leg 5 are characters shared in common with the 2 species whose development is completely known. But in those species leg 5 is long and styliform even in Stage III, while it is only in Stage IV that the appendage becomes functional in the present species. Further, in the last stage in which leg 4 is also functional, the pair of setæ carried by the penultimate segment are not so large as in the other species. Such short setæ are shown by another species also, though it has not been definitely ascribed to *Periclimenes* (Pontoniinæ, sp. 2, Terra Nova collection, Gurney, 1924). Except these 2, most of the others that have been referred to Pontoniinæ show this arrangement of setæ in leg 4.

The structural differences shown by the larvæ of the various species of *Periclimenes* known so far would therefore seem to indicate that the species composing the genus as it is constituted at present, are not so closely related as one would expect. Further, some of the differences which the present species exhibit, that of the fourth leg in particular, seem large enough to render its position within the genus somewhat doubtful.

Addendum

Gurney and Lebour have given a brief description of a single larva in the last stage and the succeeding post-larval stages derived from it (*Jour. Linn. Soc. Lond.*, 1941) and have referred them provisionally to *Periclimenes* (*Periclimenes*) *longicaudatus* (Stimpson). Since no reference to it has been made earlier it may be pointed out here that except in size and some minor characters this larva resembles closely that of *P. indicus*.

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A STUDY OF COTTON GROWN UNDER CONSTANT SOIL MOISTURES

Part I. Plant Growth and Transpiration along with Meteorological Factors

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1. Introduction

Cotton is perhaps the most important money crop in several parts of India and is grown under a wide range of conditions. The importance of increasing the yield of cotton particularly in the rainfed tracts of peninsular India where yields are low is obvious. In order to discuss agronomic measures calculated to improve yield, a study of the optimum conditions for growth of the cotton plant and a comparison of the response of different varieties to various environmental conditions are of fundamental importance. Such a study is expected to indicate the limiting factors for growth in the environment and possible means of remedying them. It will also provide an explanation why one variety succeeds in a given environment while another does not.

The main factors governing the plant growth are (1) soil moisture, (2) available nutrients in the soil and (3) weather conditions. Of these, the first two are to a large extent interdependent and are controllable under irrigation but not so in the rainfed tracts. Under both conditions it is still necessary to get information on the optimum moisture level in the soil for the satisfactory growth of the plant. When irrigation facilities are available, soil moisture can be adjusted but under rainfed conditions also measures can be found for a better conservation of available moisture or for draining away of the excess. The greater part of the living plant is made up of water and once the water content falls below a critical level in the cell sap, the tissues lose their turgidity and plants wilt and ultimately die. Again, the translocation of the minerals from the soil to the plant is brought about through the medium of soil moisture. The soil water with the nutrients dissolved in it enters the fine root hairs and is lost from the plant as transpiration from

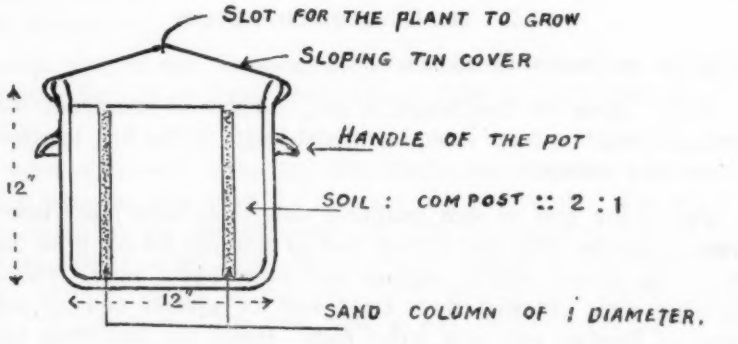
* The data discussed in this paper were collected by the author when he was a research worker at the Institute of Plant Industry, Indore, C.I., in the year 1944–45.

the surface of the leaves. The amount of water transpired is mainly dependent upon the assimilating surface of the plant and the prevailing weather condition. The present investigation was undertaken to determine the effect of different levels of soil moisture on the growth and yield of cotton and transpiration.

In Central India cotton is grown as a rainfed crop and the predominant type cultivated is Asiatic (*G. arboreum* L. var. *neglectum* forma *bengalensis*). Improved strains of this type such as Verum 434 and Jarilla are grown in Central Provinces and Malvi 9 on the Malwa plateau. A certain amount of American cotton (*G. hirsutum* L.) has also been grown in recent years under the name *Buri* in Central Provinces and *Malwa upland* in Malwa. Attempts to evolve improved strains of the American type and to extend its cultivation are in progress. The experiment, therefore, included one improved strain of Asiatic (strain 43 of *G. arboreum* L. var. *neglectum* forma *bengalensis*) and one improved strain (M.U.$_4$ of *G. hirsutum* L.) of the local upland.

2. Material and Method

Glaze earthenware pots 12″ in height and 12″ in diameter, ordinarily used in pot culture experiments, were uniformly filled after closing the hole at the bottom, with about 50 lbs. per pot of a mixture of local black soil and compost in the proportion of 2: 1. Care was taken to see that packing the soil in the pots was as uniform as possible. To help in the quick and uniform distribution of the water in the pot, two columns of sand 1″ in diameter were provided and water was added to the pot through these sand columns. The pots were suitably covered with two semicircular tin sheets so that the rain water may drain off easily. (Please refer to diagram given below.) The covers were painted white to help in reflecting the sun's rays and prevent undue heating up of top soil.



The maximum water-holding capacity of the packed soil to which compost had already been added was found to be 32% of its own weight. The experiment consisted of three levels of moisture, the one corresponding to the maximum, another half of the maximum (16%) and the third midway between the two, namely 24% with twelve pots under each level. The calculated amount of water to give these moisture levels were added a week prior to sowing so that water may get homogeneously distributed. Of the 12 pots under each level of moisture, 5 were sown with the Asiatic cotton, 5 with the American, and the remaining two pots were retained as control. To get plants as uniform as possible selfed seeds of a single plant of the strains used were first soaked in water for 36 hours and then sown three to each pot just half an inch below the surface on 13–7–1944. After the germination was complete, seedlings were thinned to one per pot. The slot in the tin covers accommodating the plant was well padded with cotton wool to prevent the tin edge from cutting the stem. The following observations were commenced when the plants were 32 days old and continued at weekly intervals:—

(1) Height of plant in cm.
(2) Number of fully developed leaves.
(3) The amount of water transpired.
(4) Evaporation as recorded by Piche evaporimeters.
(5) Total hours of bright sunshine, and
(6) The maximum and minimum temperatures.

Care was taken to space out the pots properly and maintain their relative positions to one another, as well as to the direction of sun's rays. The moisture levels in the pots were maintained by periodically adding the amount of water transpired.

3. The Developmental Study

(*a*) *Height and rate of elongation*

Table I gives the final height in cm., number of internodes, the mean internodal length and the mean internodal length of the first ten internodes in American variety.

Out of five pots in each treatment only three were taken into consideration since the other two in each level of moisture did not grow normally due to top borers, Jassids, Aphids and other insect pests. The critical differences were obtained from error sum of squares coming from six degrees of freedom and were rather high. Hence the differences were not

TABLE I

Mean final height, number of internodes, internodal length and internodal length of the first 10 internodes of three plants under each level of moisture in American variety

Characters	Moisture level			Critical difference (at 5% level of significance)
	16%	24%	32%	
Mean final height in cm. ..	45·7	53·0	60·3	8·7
Mean No. of internodes ..	14·7	15·3	17·3	1·6
Mean internodal length in cm. ..	3·12	3·45	3·48	0·41
Mean internodal length of first 10 internodes in cm. ..	3·1	3·4	3·3	0·37

brought out clearly. The results, however, gave a fair idea of the behaviour of plants under these varying conditions.

The mean differences between the final height of the American variety grown under 16% and 24%, 24% and 32% soil moistures are exactly equal but falls short of significance. This may mean that within the range of moistures employed, height increases directly as the moisture content of the soil. As the height is made up of two components (i) total number of internodes and (ii) mean length of internodes, these components of height are given in the second and third rows in Table I. Though 16% moisture had produced nodes at shorter intervals there is no significant difference in the mean internodal lengths but mean number of internodes in 32% differs significantly from 16% and 24%. With a view to find whether there is any difference at least in the early formed internodal lengths, the mean of the first ten internodes are shown in the last row. Then also there were no significant differences.

A similar analysis was, however, not possible in Asiatic variety as many of the plants especially at higher levels of soil moisture got topped due to high winds.

The relative increase in height.—The height measurements were taken every week from the time when the plants were a month old. The growth in height ceased by the eleventh week after the measurement was started in American variety at all levels and in Asiatic variety grown under 16% moisture; while in Asiatic variety under 24% moisture the growth continued for a month more. The only plant in 32% moisture was topped by high winds when it had grown 82·5 cm. in height (seven weeks after starting

the measurement). Fig. 1 gives the mean height in cm. of plants at weekly intervals of American and Asiatic varieties.

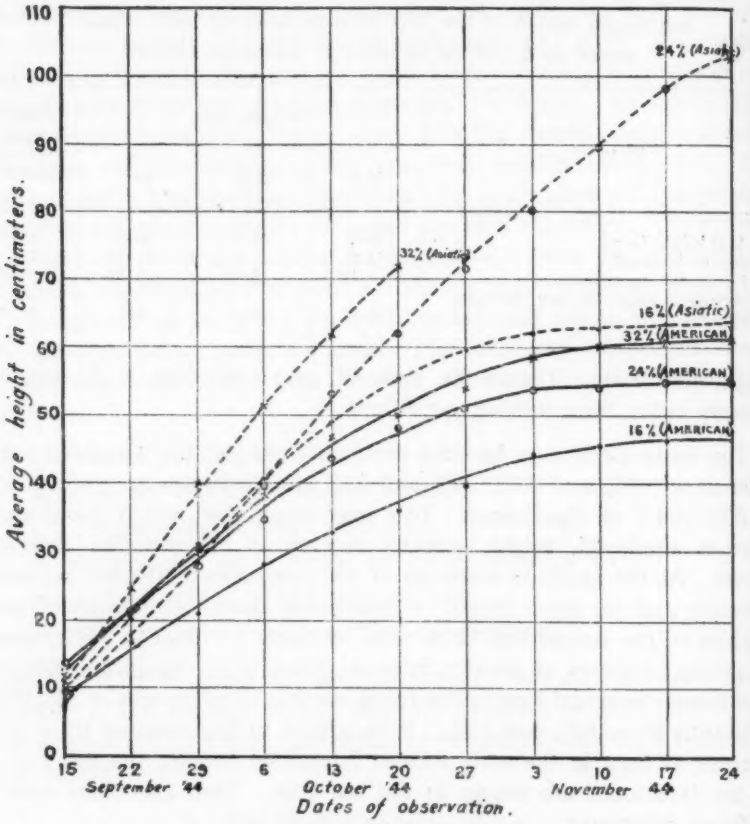


FIG. 1. Curves showing the heights in American and Asiatic plants grown under different moisture levels against time

The height curves of American variety grown under the three levels of moisture were studied by fitting (*a*) exponential curves and (*b*) quadratic curves of the second degree. The exponential equation used to fit the height curves given in Fig. 1 is $H = K - 1/e^{(b+ax)}$, where H = height in cm., K the final potential height of the plant (the upper asymptote of the exponential curve), *e* the base of the Naperian logarithms, *b* and *a* are constants and *x* time in weekly units from the commencement of observations. The quadratic curves were fitted by the orthogonal polynomial method and subsequently transformed to the equation of the form

$Y = a + bx + cx^2$. In order to bring out the relative efficiencies of these two methods, the ratios of the residual sum of squares to the total sum of squares were worked out. The following are the results obtained by these two methods:—

Level of moisture	The exponential curve $H = K - 1/e^{(b+ax)}$				Quadratic curve $Y = a + bx + cx^2$			
	K	*a*	*b*	R.S.S. / T.S.S.	*a*	*b*	*c*	R.S S. / T.S.S.
16%	52·2313	·20145	4·58604	·0105	36·646	3·813	−·351	·0036
24%	57·7648	·30003	4·13622	·0229	46·955	4·266	−·34	·0043
32%	64·4526	·28552	4·33189	·0142	50·200	4·955	−·56	·0017

R.S.S. Residual sum of squares. T.S.S. Total sum of squares.

The maximum heights that can be attained by the plants are given by K values of the exponential formula. The plants under 16% show the least K value and the K values increase linearly with higher moistures. The higher values of '*a*' for cotton grown under 24% and 32% soil moistures indicate that the growth-promoting or accelerating factor is higher in these two treatments.

The linear coefficients of the quadratic curves indicate that the growth rate is greater in the higher levels of moisture and further that the cessation of growth as indicated by *c* the quadratic component is more abrupt in 32% moisture. The proportion of the initial variate accounted for by the quadratic curve as shown by the ratio of the remainder sum of squares to the total sum of squares is very much greater than that removed by the exponential curves. This may mean that the ordinary quadratic curves give a better fit to the observations than the empirical exponential formula. It may be borne in mind that the measurements of height were started only when the plants had begun to elongate and not right from the time of germination.

(*b*) *Leaf*

The mean length, breadth, length to breadth ratio, thickness of the largest leaf and maximum number of leaves are given in Table II. The mean thickness of the leaf was determined by measuring correct to 0·01 mm. with a special kind of micro-gauge at twelve places on the leaf, taking care to avoid leaf veins.

TABLE II

The mean dimensions and the mean of maximum number of leaves

Details	American (M.U.$_4$)				Asiatic (Strain 43)			
	16%	24%	32%	C.D.*	16%	24%	32%	C.D.*
1 Length of the largest leaf in cm. ..	12·0	13·7	13·9	0·92	8·2	9·5	9·7	0·83
2 Breadth of the largest leaf in cm. ..	16·3	18·3	20·1	1·74	11·0	11·8	12·1	0·89
3 Length to breadth .. ratio ..	0·75	0·74	0·70	0·08	0·75	0·80	0·80	0·80
4 The mean thickness in mms. ..	0·25	0·22	0·20	0·014	0·29	0·31	0·21	0·026
5 Maximum number of leaves ..	44	73	84	21·3	57	127	102	19·8

* C.D. means critical difference at 5% level of significance.

The shape of the leaf, which is a varietal character and hence is not expected to change, does not differ as brought out by the length to breadth ratio.

In both American and Asiatic varieties the length of the leaf increases with the increase in soil moisture but the increase is significant only between 16% and higher levels. The difference in breadth of the leaf in both varieties is significant between 16% and 32%. The differences between 16% and 24%, 24% and 32% are not significant in the case of Asiatic variety while in American variety they are just significant. On the whole, it may be said that with increasing soil moisture bigger sized leaves are produced.

The study of the leaf size shows clearly how the plants can adapt themselves to their environment, by having larger and thinner leaves which enable them to get rid of the excess water available in higher soil moistures. It was also observed that the plants grown under 32% moisture had pale yellow leaves and these suddenly turned red at the final stages in American variety. The yellowing of the leaf may be due to saturation moisture level and lack of drainage. As regards the maximum number of leaves it is significantly less in 16% than 24% and 32% in American; in Asiatic maximum number of leaves fall significantly from 24% to 32% and 32% to 16%.

The leaf number was recorded weekly along with other plant characters. Table III below gives the analysis of variance for number of leaves until 5–1–1945 when a cold-wave set in with the minimum temperature touching 33° F. and the leaves started shedding.

TABLE III

Analysis of variance for leaf

Due to	D.F.	Mean Square					
		American (M. U.$_4$)			Asiatic (Strain 43)		
		16%	24%	32%	16%	24%	32%
1 Pots ..	2	590·1†	144·1†	629·0*	306·4†	709·2†	476·6†
2 Dates ..	16	352·5†	1000·9†	1331·3†	801·6†	5331·5†	2594·3†
(*a*) Linear component	1	2467·8†	8624·2†	7323·6†	8725·4†	72358·6†	26625·3†
(*b*) Quadratic component ..	1	3026·8†	6572·2†	13292·3†	3789·6†	7919·3†	12481·1†
(*c*) Cubic component	1	0·0	117·9	4·9	37·8	3046·5†	1292·0†
(*d*) Remainder ..	13	11·2	53·9	52·6	21·0	129·2	82·3
3 Error ..	32	8·91	18·89	52·77	16·12	32·20	27·61

* Significant at 5% level. † Significant at 1% level.

The errors sum of squares for different moisture levels are not of the same order and hence combined analysis is not justified. The pot to pot variation is significant in all cases. This indicates that the technique of preparing the pots needs modification. It is possible that the soil was not packed sufficiently uniformly. Sieving the soil and compost before filling might remove to certain extent pot to pot variation.

The date mean square is highly significant in all cases. This is what we should expect as the leaf number was continuously increasing up to a certain date when shedding commenced. A polynomial of the third degree was fitted and the mean squares for (*a*) linear, (*b*) quadratic, (*c*) cubic components and (*d*) the remainder are given in Table III. The cubic mean squares are significant only in Asiatic variety grown under 24% and 32% moisture levels.

The following are the coefficients of linear, quadratic and cubic components of the formula $Y = a + bx + cx^2 + dx^3$:—

Moisture level	American (M.U.$_4$)			Asiatic (Strain 43)			
	a	*b*	*c*	*a*	*b*	*c*	*d*
16%	95·059	4·260	−1·082	146·74	8·01	−1·211	..
24%	145·882	7·963	−1·595	263·95	34·60	−1·751	−0·2682
32%	164·765	7·338	−2·268	233·75	19·61	−2·198	−0·1305

Fig. 2 gives the number of leaves in both American and Asiatic varieties with march of time and the curve of best fit for leaf number.

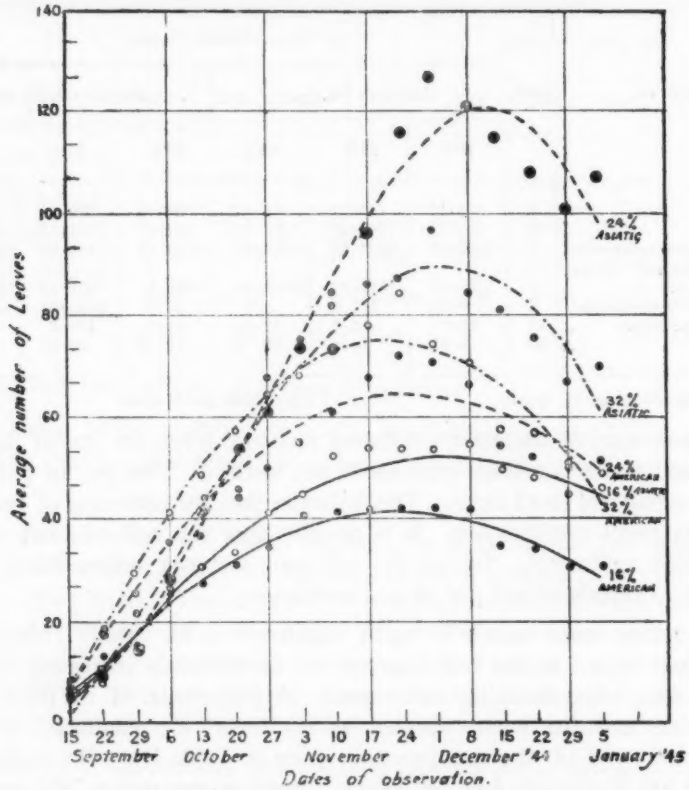


FIG. 2. Average number of leaves in American and Asiatic plants grown under different levels of moisture, on different dates

In American variety the rates of development of leaf in 24% and 32% are greater than in 16% and so too the shedding as given by *c* coefficients. In Asiatic variety the rate of leaf development is greatest in 24% while the shedding of leaf increases with the increase in soil moisture. The negative values of cubic coefficients *d* in 24% and 32% moisture levels in Asiatic variety may mean that (i) leaf development in the initial stages and (ii) leaf shedding at the close of the period are accelerated to a certain degree.

(*c*) *Boll and seed development*

For both American and Asiatic varieties, the opening of the first flower in plants under different treatments was found to take place more or less

simultaneously. We shall briefly examine here the number of bolls produced under different treatments, the locular composition of boll, the average weight of the seed cotton per boll, the average number of mature seeds per boll and the total yield of kapas. Table IV gives the results.

TABLE IV

Shows mean number of bolls produced, locular composition of boll, average number and weight of seed cotton per boll and total yield of kapas

Details	American (M.U.$_4$)				Asiatic (Strain 43)			
	16%	24%	32%	C.D.*	16%	24%	32%	C.D.*
1 Mean total No. of bolls per plant ..	10	18	14	7·2	18	27	19	7·9
2 Mean No. of bolls burst before 2–2–45 ..	7·3	11·3	11·3	3·8	12·7	17·0	10·0	7·6
3 Average No. of mature seeds per boll ..	33·2	34·4	37·0	4·2	16·6	15·9	18·0	3·1
4 Average weight of seed cotton per boll in gm. ..	5·20	5·49	5·39	1·01	1·49	1·76	1·47	0·63
5 Locular composition No. of 4 locules : 5 in American and 3 locules : 4 in Asiatic ..	0·81	1·21	1·37	..	0·50	0·78	0·66	..
6 Total yield of kapas in gm. ..	48·6	96·4	72·4	34·08	23·3	40·2	18·5	9·78

* C.D. means critical difference at 5% level of significance.

The total number of bolls per plant in Asiatic variety is significantly higher in 24% than other moisture levels while in American this is significantly higher in 24% than 16% only. Also in American the number of bolls that opened before 2–2–45 is significantly less in 16% than in higher levels. But the differences in average weight of seed cotton per boll and number of seeds per boll are not significant. It may be mentioned here that the average weight of a single American seed with lint and fuss on was of the order of 0·16 gm. This high value may be due to the rich soil to which compost was added, lower number of bolls per plant and steady rate of moisture supply. As regards the total yield of kapas for both varieties, 24% moisture level appears to be the best and the order of merit in American is 24%–32%–16% and in Asiatic 24%–16%–32%. Ordinarily bolls in American have four locules while those in Asiatic have three. With the increase in soil moisture the number of five locular boll seems to increase in American whereas in Asiatic 24% moisture had produced the largest number of 4-locular bolls. But in general the bolls and seeds from Asiatic

variety under 16% and 32% moisture levels were small and the seeds and fibres appeared to be immature. To bring out the contrast in plants grown under different moisture levels, photographs were taken of typical American and Asiatic plants grown under different levels of moisture when the plants were just four months old. The photographs I and II show American and Asiatic plants respectively and the soil moisture levels are marked on the pots. A comparative study of heights may be made with the help of the scale in feet shown in the photographs.

Transpiration Study

On days when leaf number and plant height were recorded, the pots were weighed on a sensitive platform balance and the weight of water lost was made up by adding water correct to 10 c.c. On hot days, with the knowledge of the previous week's transpiration, the approximate amount of water that should have transpired was added on alternate days. The amount of water supplied during the whole week minus the quantity of water added to the control pots may be considered approximately equal to the water transpired by the plant.

The mean amounts of water transpired per day in c.c. during the period of 147 days from the time when plants were one month old were as follows:—

Cotton (Variety)	American (M.U.$_4$)			Asiatic (Strain 43)		
Soil moisture level ..	16%	24%	32%	16%	24%	32%
Mean amount of water transpired per day in c.c. ..	338	687	856	161	389	289

American variety grown in saturated soil moisture transpired an enormous amount of nearly a litre of water in a day and this is nearly 2½ times the amount of water transpired by plants under 16%. In Asiatic transpiration was maximum in 24% soil moisture. A comparison of the intake of water by American and Asiatic plants grown under the same level of soil moisture tells us that American variety requires two to three times the amount of water utilised by Asiatic variety. Fig. 3 gives the additive totals of water transpired. The curves are quite sigmoid in form. The rate of transpiration as indicated by the point of inflexion of the curve is maximum on the date of maximum number of leaves. After the opening of the first boll the transpiration rate falls considerably. The opening of the first boll happened to coincide with the cold wave and the fall in transpiration may well be ascribed to the low temperature and shedding of leaves.

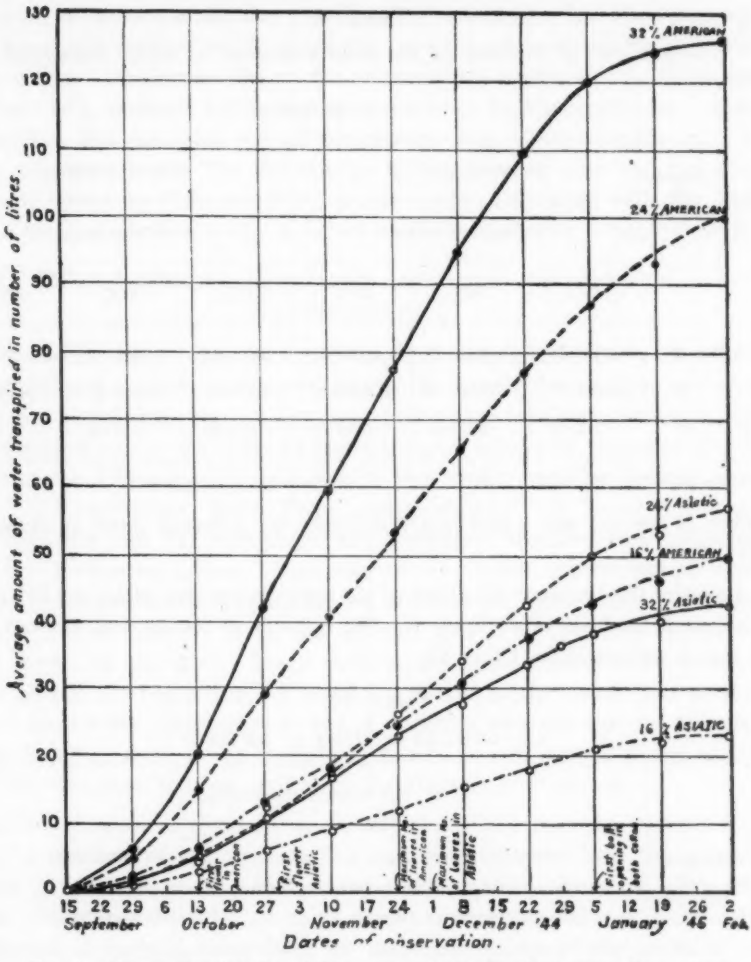


FIG. 3. Curves showing the additive amount of transpired water from plants grown under different moisture levels with march of time

The analysis of variance of the weekly amount of water transpired is given in Table V.

The insignificant pot to pot variations in 24% moisture in American and 32% in Asiatic suggest that plants under these levels of moisture transpired in a similar fashion. In all levels of moisture the differences in transpiration on different dates are highly significant. This is what we should expect since one of the main factors governing transpiration, namely the

TABLE V

The analysis of variance of the weekly amount of water transpired

Due to	Degrees of freedom	Mean square					
		American (M.U.$_4$)			Asiatic (Strain 43)		
		Levels of moisture			Levels of moisture		
		16%	24%	32%	16%	24%	32%
Pots ..	2	15230·7†	3759·6	12853·3*	2669·4†	5410·7†	688·1
Dates	16	31564·8†	105415·3†	285118·4†	8579·1†	64566·2†	34303·6†
Error	32	740·14	2730·51	2629·51	180·20	266·08	675·27

* Significant at 5% level. † Significant at 1% level.

number of leaves, was significantly different on different dates as shown in Table II.

In order to eliminate the effect of the varying number of leaves on dates the adjusted analysis of variance for the number of leaves was worked out, and this is furnished in Table VI.

TABLE VI

The adjusted analysis of variance

Due to	Degrees of freedom	The adjusted mean square					
		American (M.U.$_4$)			Asiatic (Strain 43)		
		Moisture level in soil			Moisture level in soil		
		16%	24%	32%	16%	24%	32%
Pots ..	2	1482·7	767·8	1807·7	1498·8*	1521·8*	825·8
Dates	16	9985·6*	32306·6*	88490·7*	2006·1*	11133·1*	14283·7*
Error	31	729·5	2685·4	1489·3	185·8	264·7	651·4

* Significance at 1% level.

In American the pot variances have become insignificant. It may be inferred that the variation was mainly due to differences in the number of

leaves of plants grown under the same level of moisture. But the variances due to dates still remain highly significant in spite of considerable decrease in their values. In Asiatic variety the adjusted pot variances due to plants at 16% and 24% moisture levels, still continue to be highly significant. Also the variances due to dates for all treatments are highly significant. A possible explanation for this persistence of significance may be that the number of leaves on different dates is not highly correlated with the leaf area and the transpiration under different weather conditions is not the same.

5. The Atmospheric Conditions and Their Influence on Transpiration

In the transpiration study it was found that even after making an allowance for differences in the number of leaves, the weekly fluctuations in transpiration are significant. It is a common knowledge that on drier days, the plant transpires more. In order to assess the part played by the atmospheric conditions, the following observations of the weekly weather factors were made:—(1) Evaporation from Piche evaporimeters, (2) Total hours of bright sunshine and (3) Maximum and minimum temperatures recorded within the Stevenson screen. Two sets of six Piche evaporimeters were hung inside the pot-house surrounded by experimental pots at the following heights:—18″, 36″ and 54″ from the ground, that is 6″, 24″ and 42″ above the tin covers of the pots. Mean evaporation from these heights is used in working out the regression. The weekly means of the above four meteorological factors are represented in Fig. 4, together with the amount of water transpired during these weeks from both American and Asiatic plants grown under 24% moisture level as typical of the transpiration curves.

From a perusal of these curves it will be noticed that the evaporation, which is a function of saturation deficit and wind seems to have the greatest influence on transpiration. Partial regression coefficients with (i) b_1 the number of leaves and (ii) b_2 the mean evaporation from Piche evaporimeters as independent variates were fitted to the mean values of transpiration on different dates. Table VII gives the partial regression coefficients of number of leaves and evaporation as recorded by Piche evaporimeters.

The regression coefficients (b_1) of number of leaves on transpiration are highly significant for all treatments except for 32% moisture treatment in Asiatic where it is significant only at 5% level. The partial regression coefficients (b_1) for different moisture levels do not differ significantly. The partial regression coefficients (b_2) of the effect of evaporation are also highly significant in all cases except in Asiatic under 16% moisture level. The evaporation seems to have a progressively increasing influence on the rate

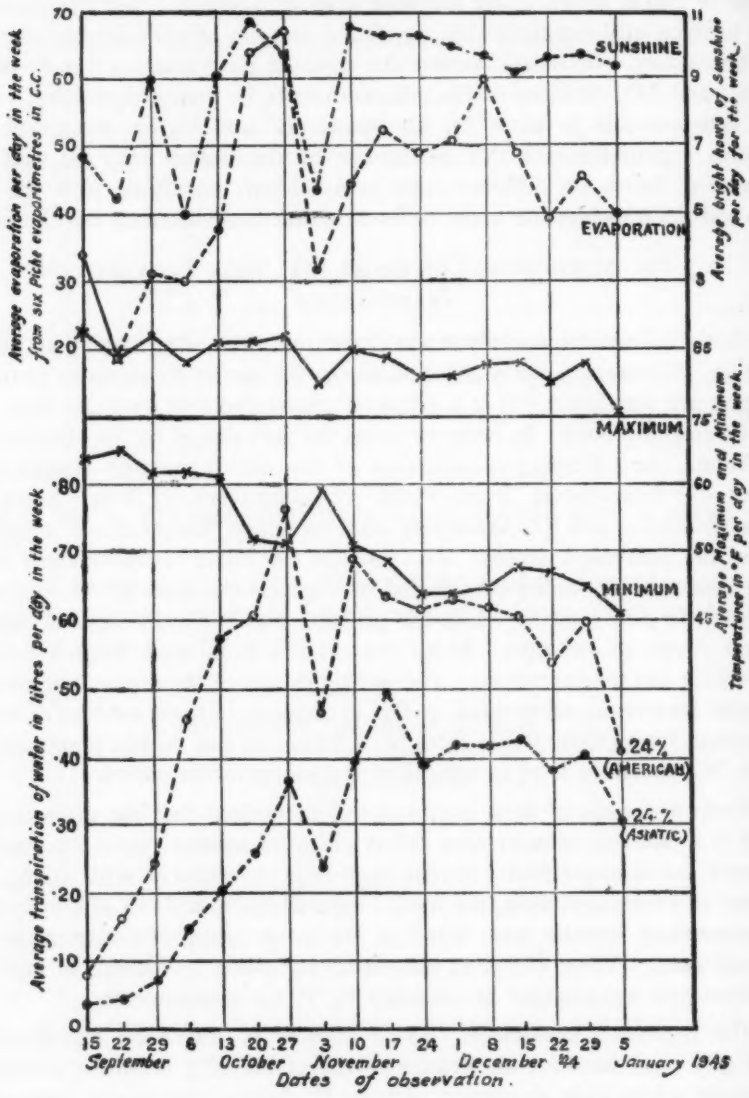

Average evaporation per day in the week from six Piche evaporimetres in c.c.
Average bright hours of Sunshine per day for the week.
SUNSHINE
EVAPORATION
MAXIMUM
MINIMUM
Average Maximum and Minimum Temperatures in °F per day in the week.
Average transpiration of water in litres per day in the week
24% (AMERICAN)
24% (ASIATIC)
15 22 29 6 13 20 27 3 10 17 24 1 8 15 22 29 5
September October November December '44 January 1945
Dates of observation.

FIG. 4. Curves indicating the maximum and minimum temperatures, average number of hours of bright sunshine, evaporation from Piche evaporimeter together with the average amount of transpiration per week from plants grown under 24% soil moisture

TABLE VII

The partial regression coefficients of number of leaves and evaporation

Details	American (M.U.$_4$)			Asiatic (Strain 43)		
	Moisture level in soil			Moisture level in soil		
	16%	24%	32%	16%	24%	32%
1 Partial regression coefficient b_1 of number of leaves on transpiration ..	5·1469†	5·7398†	7·9203†	2·3762†	2·5833†	1·5617*
2 The standard error of b_1 ..	1·2922	1·4053	2·1634	0·4554	0·3561	0·5669
3 Partial regression coefficient b_2 of evaporation on transpiration ..	3·7935†	6·7257†	10·9990†	1·0964	3·5097†	4·6559†
4 The standard error of b_2 ..	1·0948	2·0063	3·5621	0·5818	1·1733	1·3029

* Significant at 5% level. † Significant at 1% level.

of transpiration in both American and Asiatic varieties grown under increasing levels of moisture, although the differences between the b_2 regression coefficients of 16% and 24%, 24% and 32% in American and 16% and 24% in Asiatic fall just short of significance. The b_2 partial regression coefficient of plants grown under 32% level of moisture is significantly higher than for plants under 16% moisture in both the varieties. It may be concluded that the positive response of transpiration to the atmospheric dryness is greater from plants grown under higher soil moistures. The reduced response of plants growing under lower moisture levels to the saturation deficit and wind is probably brought about by some or all of the following leaf mechanisms:—

1. The lesser number of stoma and the smaller size of the opening.
2. The reduced leaf size.
3. The increased leaf thickness.
4. The lesser number of spongy parenchyma.
5. The greater hairiness and waxy coating on either surfaces of the leaf.

We have already seen how by adjusting for the leaf number, we are able to significantly reduce the variation due to transpiration. Table VIII gives the original sum of squares for dates, sum of squares due to regression

function of the independent variates, namely the number of leaves and evaporation, residual sum of squares for dates, residual mean square for dates and residual error mean square, with their respective degrees of freedom.

TABLE VIII

The double adjusted analysis of variance for dates

Details	D.F.	American (M.U.$_4$)			Asiatic (Strain 43)		
		Moisture level in soil			Moisture level in soi		
		16%	24%	32%	16%	24%	32%
1 Original sum of squares for dates	16	505,036	1686,644	4561,894	137,266	1033,059	548,857
2 Sum of squares of regression function	2	424,258	1412,423	3773,206	114,748	968,103	430,369
3 Residual sum of squares for dates	14	80,778	274,221	788,688	22,518	64,956	118,488
4 Residual mean square for dates	14	5,770†	19,587†	56,335†	1,608†	4,640†	8,464†
5 Residual mean square for error	31	1,730	2,685	1,489	186	265	651

† Significant at 1% level. D.F.= Degrees of Freedom.

By adjusting the sum of squares for differences in number of leaves and evaporation on different dates, we have reduced this amount by more than 80% in most of the treatments. Alternately the multiple correlation between transpiration and number of leaves and evaporation, which is merely the square root of the regression sum of squares over total sum of squares (Fisher, 1932) is of the order of 0·9 and is highly significant. It is however interesting to note that the residual sum of squares for dates are highly significant even after eliminating the influence of these two factors on transpiration. It is probable that the residual mean square would have reduced still further, if, instead of number of leaves, the total assimilating surface were measured along with the mean thickness of the leaf lamina. The possibility of other external factors like maximum and minimum temperatures of the atmosphere and bright hours of sunshine affecting the transpiration is not ruled out.

6. Discussion and Conclusions

The growth of a plant is controlled by its genetic constitution or the internal factor and the external factors mainly moisture, air and nutrients in soil and weather conditions under which it develops. To evaluate the

effect of some of the external factors on plant growth and yield, it is essential that the remaining external factors should not be altered.

For growth and transpiration study of plants grown under different levels of soil moisture, we have to resort to pot cultures where it is possible to account for water lost by transpiration and evaporation and water lost as run of and gravitational water under field conditions can be avoided.

Such experiments carried out under natural conditions are rare. Leather (1910) has made pioneer research in India with such open pots. Briggs and Shantz (1911) devised the "wax seal" technique which came into vogue with most of the later pot studies. In adopting this technique, it is probable that the constitution of soil air is upset and this may more than offset the gain in checking direct evaporation from the soil. In the present study the pots were filled up with mixture of ordinary soil and compost (no ingredient being sieved) and covered with pairs of semi-circular tin lids painted white. This method was found to be quite satisfactory provided there are suitable number of dummy pots as control.

In American plants the relative increase in height was more in plants grown under higher levels of moisture. The cessation of elongation also was more abrupt in plants under higher moisture. The final heights of plants under these three levels of moisture, *viz.*, 16, 24 and 32% were found to increase in a linear fashion with higher moisture levels. This is in agreement with the findings of Jaganatha Rao (1938).

To the weekly development in height data, polynomials of the 2nd degree and exponential curves were fitted. Polynomials gave a much better fit than the exponential formulæ. It was pointed out that measurements were started rather late when the plants have already started to elongate. Heath (1932) experimenting with rain-fed cotton in South America found that an empirical exponential formula of the form $H = Ae^{bT}$ gives a very good fit to the height data collected right from the time of germination to a stage when plants have attained a good height. Similar results were obtained by Afzal and Iyer (1934) for irrigated cotton in Punjab and Crowther (1944) for irrigated cotton in Egypt but not for irrigated cotton in Sudan.

In all these studies, the height measurements were not continued to that stage when the effect of growth-inhibiting factor gains the upper hand. In the present study, unfortunately observations soon after germination were not started but were started only when the plants were 10 to 12 cms. high. Hence an exponential expression of the form $H = K - 1/e^{(b+ax)}$ was found to be more appropriate. As early as 1907 Robertson (1907 to

1908) advanced the view that growth is of the nature of a mono-molecular autocatalystic reaction. A reaction of this type proceeds slowly at first, increases rapidly to a maximum and then declines rapidly. The curve of such a reaction is thus typically sigmoid in shape. In other words, the growth at any stage is a function of the growth which it has already attained as well as the residual growth. The residual growth is merely the maximum growth that a plant can make minus the growth which it has already made. Such a biological phenomena may be represented by the general expression

$$Y = I + \frac{K}{1 + e^{f(t)}},$$

where Y is the magnitude of the character at any time, I the lower asymptote, K the upper asymptote, e the Napierian logarithm and $f(t)$ a function of time. The $f(t)$ may be represented as a polynomial $a + bt + ct^2 + dt^3$, etc. Such an expression has been used to fit the Donaldson's (1915) data on the growth in weight of the male white rat. Ramabhadran (under publication) working with the data on height of rice plant found that the above expression is quite appropriate. Probably the law given by the expression may hold good in the case of cotton plant also.

The number and size of leaf increased with the increase in moisture in American while in Asiatic the number of leaves in plants under 32% moisture level was lower than that in plants in 24% moisture level. The average thickness of the leaf lamina steadily fell in American variety with increase in moisture level but in Asiatic there was a significant fall in leaf thickness only in plants under 32% moisture. This is not in agreement with Henser's (1915) conclusion, who working with wheat, found that though the dimensions of the leaf increase with additional moisture, the thickness remained the same.

Albert and Armstrong (1931) observed that percentage of oxygen goes down and the percentage of CO_2 is greater in soil with higher moisture content. The saturated soil moisture or the increase in CO_2 might have led to the yellowing of leaves in both the varieties and subsequent reddening in American. Dastur (1941 and 1942) working with Punjab-American found that the leaves of plants affected by *tirak* turn pale green, yellow and finally red before they are shed. The *tirak* may be caused by nitrogen deficiency in plants or the presence of sodium salts in abnormal amounts in sub-soil. As the soil in pots was quite rich in nitrogen and also free from salts both the reasons may be ruled out in this experiment. It may be added here that in American certain abnormalities in boll and lint development were noticed.

A short note of these abnormalities has appeared in *Current Science* (Sreenivasan, 1945).

In American variety the transpiration increased with the increase in moisture level and the transpiration in Asiatic variety was maximum in 24% moisture. The leaf number and evaporation from a free surface were found to influence transpiration to a very great extent. Working with rice, Ganguly (1934) found that except for slight variation, the rate of transpiration followed closely the daily temperature, which may be highly correlated with atmospheric humidity.

Optimum moisture level for American variety is evidently higher than for Asiatic, as seen by comparing the height, number of internodes, number of leaves, average weight of seed cotton per boll, locular composition and transpiration. Thus the present study brings out clearly that American variety is not adversely affected by very high levels of moisture while Asiatic variety cannot tolerate higher levels of soil moisture. Also the total water requirement for successfully growing American is more than double the amount required by Asiatic and this should be borne in mind while spreading American in Central India.

Summary

1. The water requirement and the performance of a variety in each of American and Asiatic cotton were studied.

2. A critical study of the rate of elongation was made.

3. In American the final height, maximum number of leaves and size of leaves increased with increase in soil moisture. But the leaf thickness decreased with the increase in soil moisture (Tables I and II).

4. In Asiatic variety number of leaves and leaf thickness were maximum in 24% soil moisture (Table II).

5. In both varieties the number of bolls and yield of kapas were maximum in plants grown under 24% soil moisture (Table IV).

6. Transpiration increased with increase in soil moisture in American while in Asiatic variety the transpiration was maximum at 24% moisture.

7. The factors governing the transpiration were discussed. The main factors which influence the transpiration were found to be the number of leaves and the evaporating power of the atmosphere (Tables VII and VIII).

8. The transpiration was highly significantly related to number of leaves and evaporation and the multiple correlation 'R' was found to be of the order of 0·9.

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Photograph I. American (M.U. 4)

Photograph II. Asiatic (Strain 43)

STUDIES ON THE ANATOMY OF THE TAIL IN SAURIA AND RHYNCHOCEPHALIA*

Part III. *Varanus monitor* (Linné)

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I. Introduction

The common monitor lizard of India (*Varanus monitor*) has been selected to exemplify the undulating type of tail found in certain saurians, which is adapted to perform rapid lashing movements from side to side, and shows many interesting features hitherto undescribed. It shows an unmistakable similarity to the tails of fishes and amphibians, although the myomerism of the musculature is merely superficial and has been lost in the deeper parts. Perhaps this type, on the whole, is more primitive than the autotomous and prehensile types.

II. General

In *Varanus monitor* the length of the tail, as might be expected from its function, exceeds that of the trunk. The author measured it in numerous individuals of all ages and both sexes and found it to be usually one and a half times the length between snout and vent.

* Part of a thesis approved for the Ph.D. degree in Agra University.

The tail is somewhat depressed dorso-ventrally in its thick basal region, but undergoes a gradual lateral compression behind. At a distance of about one-third of its length from the vent, there appears a conspicuous dorso-median ridge on it which runs up to the end, although it is much less prominent near the extremity.

The presence of lateral compression in the tail in the greater part of its length, especially in the middle region, is apparently an adaptation for movements of flexion. The thick proximal part which is almost oval in transverse sections, is not capable of lateral movements to the same extent as the middle and posterior regions.

III. Epithelium

A careful study of thick hand-cut sections, both transverse and longitudinal, as well as of minute dissections, showed the following peculiarities in the caudal epithelium of *Varanus monitor*:

(1) The epithelium is not divided by lines of cleavage as in *Lacerta*, *Gecko*, *Hemidactylus* and other autotomous lizards, but is continuous throughout the extent of the tail.

(2) The skin is tough and characterized by the strongly developed dermis, which shows a compact laminated structure and immediately overlies the musculature. This appears to be an adaptive modification, since the texture and disposition of the skin is generally correlated, to a fair extent, with the physiology of the tail. In those lizards (as well as in those parts of the body) in which active movements take place, the skin is tough and is in intimate connection with the underlying tissues; while, on the other hand, in lizards with less mobile tails, the caudal epithelium is delicately developed and loosely connected with the underlying musculature. It is interesting to note that the epithelium of the regenerated tail of *Hemidactylus flaviriridis*, as observed by Woodland (1920), is tough in texture. This fact, as well as the scalation of the regenerated tail, has been regarded as a reversion to the ancestral condition.

(3) The sub-cutaneous fat layer is absent in *Varanus monitor* as in *Sphenodon punctatus* (Woodland, 1920; Ali, 1941); *Lacerta vivipara* (White, 1915), and *Mabuya dissimilis* (Sibtain, 1938).

(4) The dorso-median epithelium is very much thickened and forms the dorsal crest of the tail. In transverse sections, the dermis of this region is, on the whole, similar in appearance to that of *Sphenodon punctatus* except that there are no fat-spaces. This difference is perhaps due to the lack of degeneration which is expected in an extremely agile tail.

IV. Scalation

Except for a small part at its base where the scales are very small and irregular, the tail is almost completely covered by medium-sized rectangular and slightly convex scales with a definite arrangement. On the ventral surface, they form a sort of square system, resulting from a series of longitudinal rows crossing transverse ones. The *ventrals* have their long sides parallel to the longitudinal axis of the tail. They pass on, at the sides of the tail, into the *lateral scales* which are continued above into the crest-forming *dorsals*. Thus the tail appears to be surrounded by rings of scales. As it tapers posteriorly, the laterals diminish in length but the ventrals retain their size. Consequently, an additional transverse row of laterals makes its appearance between every two rings of scales on the lateral and dorsal aspects of the tail. In other words, in the posterior caudal region two transverse rows of ventrals correspond to three rows of laterals. All these rows are separated by grooves bearing microscopic scales, an arrangement for the extension of the skin during lateral flexions.

The ventrals are slightly imbricate, but the laterals are juxtaposed. Each bears a median longitudinal keel.

The dorsal ridge of the tail is composed of a double row of slightly recurved tubercles between which is a fine furrow running longitudinally up to the end of the tail. The tubercles become more distinct as we proceed backwards.

V. The Caudal Vertebræ

The vertebræ (Figs. 1 and 2), especially those of the midlde caudal region, show a number of structural peculiarities which are apparently adaptations for the vigorous lateral flexions of the tail. The following features are worth noting.

(1) The vertebræ are compact and strongly built. This is due to the fact that they have to withstand a great deal of stress during the violent lateral undulations.

(2) They are situated not dorsally as in *Chameleon zeylanicus*, but almost in the middle of the tail, equidistant from the dorsal and ventral surfaces (Figs. 5 and 6). The great elongation of their neural and hæmal spines results in a complete division of the right side of the tail from the left, thus forming a vertical mesial partition in the tail which is much more prominent than the horizontal myoseptum.

(3) The ball-and-socket articulation between successive vertebræ is very well developed while the centra show compression in a direction reverse

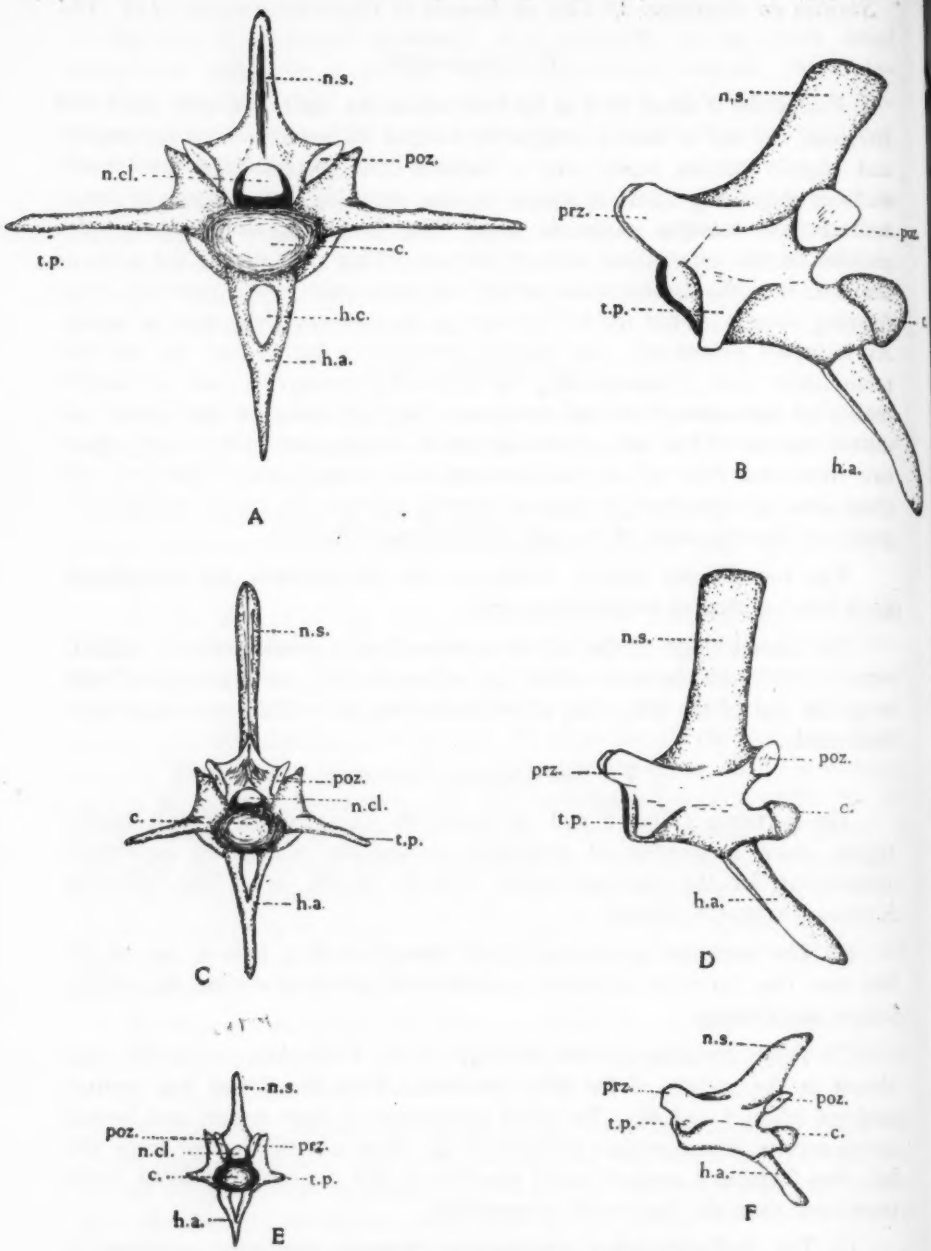

FIG. 1. Vertebræ of *Varanns monitor* : *A* and *B*, from immediately behind the sacral region ; *C* and *D*, belonging to the middle of the tail ; and *E* and *F*, from near the end of the tail. *A*, *C* and *E* — posterior views. *B*, *D* and *F*—lateral views (× 3).

c., centrum ; *h.a.*, hæmal arch ; *h.c.*, hæmal canal ; *n.cl.*, neural canal ; *n.s.*, neural spine ; *poz.*, post-zygapophysis ; *prz.*, prezygapophysis ; *t.p.*, transverse process.

FIG. 2. Lateral view of the vertebrae of *Varanus monitor* from the end of the tail (× 3). *.v.f.*, intervertebral foramen ; other abbreviations as in the previous figure.

to that of the tail as a whole. They are shortened in a dorso-ventral direction and elongated from side to side. Such a configuration not only ensures the necessary measure of strength and security of the joint, but also allows lateral movements with ease while restricting movements in the dorso-ventral direction.

(4) The zygapophyses are relatively small and may be contrasted to the extremely elongated ones found in *Chameleon zeylanicus*. This is due to the fact that the movements in a dorso-ventral direction are much limited.

(5) As compared to the extremely well developed neural and hæmal arches, the transverse processes are small and narrow. They are in the form of horizontal plates attached to the centra close to their anterior ends.

(6) The neural spine is much elongated, laterally compressed and points backwards. The hæmal arches which are nearly as long as the neural spines, are Y-shaped, with the two arms of the 'Y' much smaller than the basal stroke. The major part, approximately two-thirds of each hæmal arch, is formed by a spatulate bony rod which is laterally compressed and directed backwards. A longitudinal furrow is present in the middle line on the ventral aspect of each vertebra between the two arms of the hæmal arch.

(7) As we proceed backwards towards the end of the tail, the neural spines and the hæmal arches diminish in size until they are almost lost at the extremity. The pre- and post-zygapophyses get much reduced, the transverse processes are represented by slight elevations on the anterior parts of the centra, and the vertebræ assume the form of elongated hollow cylinders strongly joined to each other. This diminution in size, however, of the neural spines and the hæmal arches does not take place at the same ratio as that of the transverse processes, so that the posterior caudal vertebræ, as compared to the anterior ones, are higher than broad. The following table gives the relative measurements in this connection:—

The Region to which the vertebra belongs	Distance between the tip of the neural spine and the apex of the hæmal arch (= *Height of the vertebra*)	Distance between the distal ends of the two transverse processes (= *Width of the vertebra*)	Ratio between the height and the width of vertebra
Anterior caudal ..	28 mm.	33 mm.	0·85
Middle caudal ..	25 mm.	15 mm.	1·66
Posterior caudal ..	11 mm.	6 mm.	1·83

VI. The Caudal Musculature

Lying immediately underneath the epithelium are the flexor muscles of the tail which are divided into right and left sets more distinctly than into the *epiaxonic* and *hypaxonic* ones.

In correlation with such a division of the musculature into lateral sets, the vertebræ, as already described, are situated not within the dorsal half of the tail, but almost midway between the dorsal and the ventral surface. The horizontal myoseptum can scarcely be distinguished from the other connective tissue septa separating the muscle bands. A thick dorso-ventral vertical partition, composed of tough connective tissue and embracing the neural spine and hæmal arches, is developed.

After the skin is removed (Fig. 3), the caudal musculature shows a myomeric arrangement in surface view. There are five longitudinal rows of V-shaped inscriptions on each side, two being dorsal and three ventral to the mesaxonic septum. This appearance, however, is merely superficial. A careful examination of dissections and serial sections establishes the fact that this segmentation does not extend into the deeper parts of the muscles, which are not divided myomerically but disposed longitudinally. Some of them proceed for almost the whole length of the tail; others end after running for a short distance. The superficial pattern is formed by fine tendons (*inscriptiones tendineæ*) overlying the musculature.

There are three pairs of epiaxonic muscles, as described below, at the base of the tail. The second pair ends after reaching about one-third the length of tail so that in the posterior region there are only two pairs.

(1) *M. Transverso-spinalis*

These muscles occupy, as in *Chameleon zeylanicus*, the longitudinal canal-like spaces between the neural spines and zygapophyses, and are continued into the trunk. There are no tendons as in chameleon zeylanicus, but the surface of the muscle bears a series of V-shaped *inscriptiones tendineæ* with their apices pointing backwards (Fig. 3). One of the arms of the 'V' is

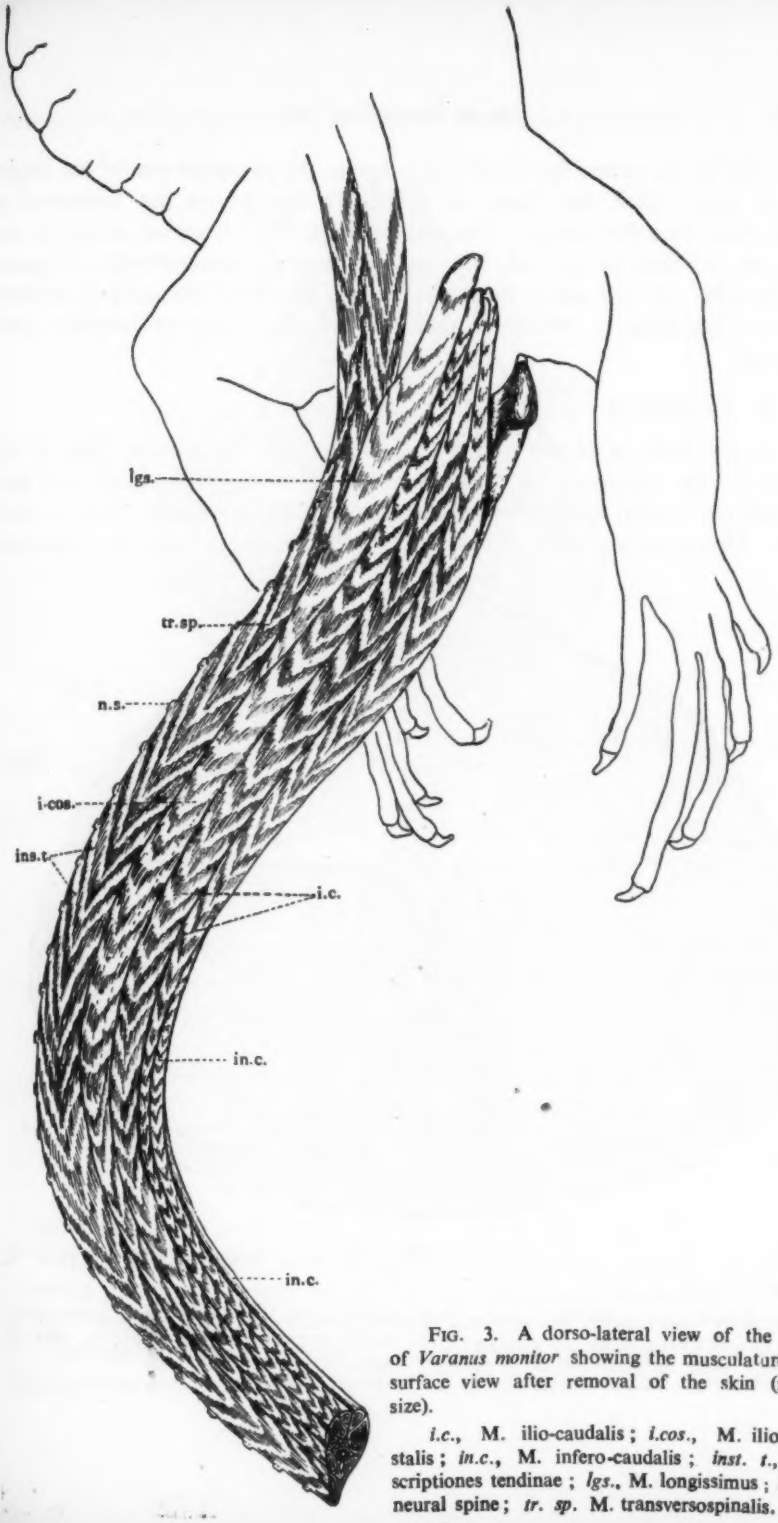

FIG. 3. A dorso-lateral view of the tail of *Varanus monitor* showing the musculature in surface view after removal of the skin (nat. size).

i.c., M. ilio-caudalis; *i.cos.*, M. ilio-costalis; *in.c.*, M. infero-caudalis; *inst. t.*, inscriptiones tendinae; *lgs.*, M. longissimus; *n.s.*, neural spine; *tr. sp.* M. transversospinalis.

attached to the neural spine and the other to the zygapophysis of the appropriate side; while the angles of successive inscriptions are connected to each other by a fine streak. The muscle (Figs. 4–6) is almost round in section at the base of the tail, but becomes laterally compressed as it passes backwards. At the same time, the neural spines of the caudal vertebræ become conspicuous structures and the tail also becomes laterally compressed.

(2) *M. Longissimus*

In the anterior region of the tail, each *longissimus* muscle (Fig. 4) lies dorsal to the transverse processes between the transverso-spinalis and iliocostalis (*vide infra*) and thus there are three epiaxonic muscles here on each side. This is an important difference from the condition found in *Chameleon*

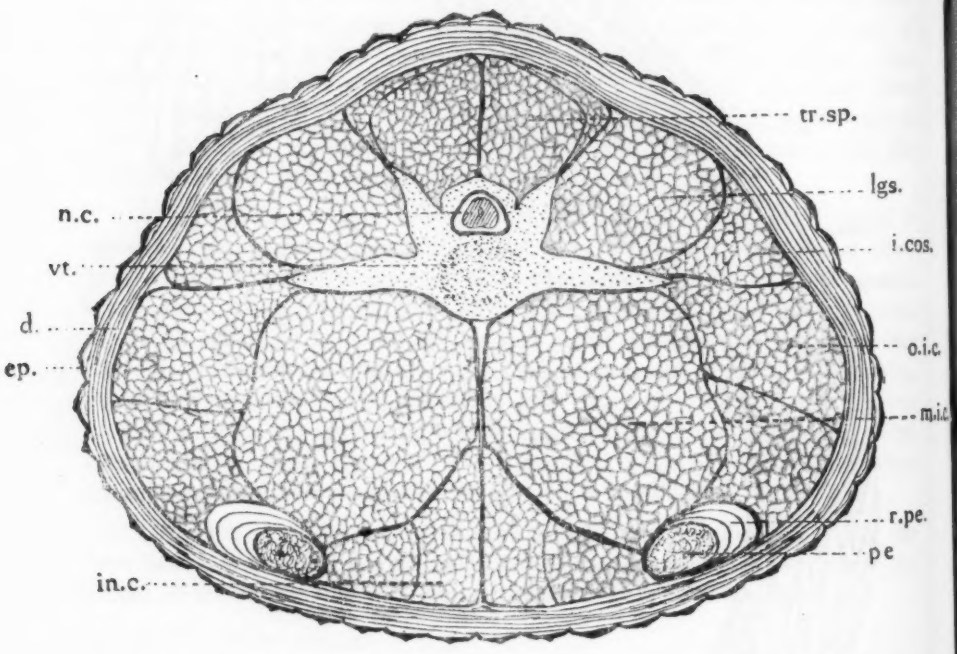

FIG. 4. Transverse section of the tail of *Varanus monitor* passing through the plane 'X' of Fig. 7 (× 2).

d., dermis ; *ep.*, epidermis ; *m.i.c.*, Pars mesialis of M. Iliocaudalis ; *n.c.*, nerve cord ; *a.i.c.*, Pars externalis of M. Iliocaudalis ; *r.pe.*, Retractor muscles of the penes ; *vt.*, body of vertebra ; other abbreviations as in previous figures.

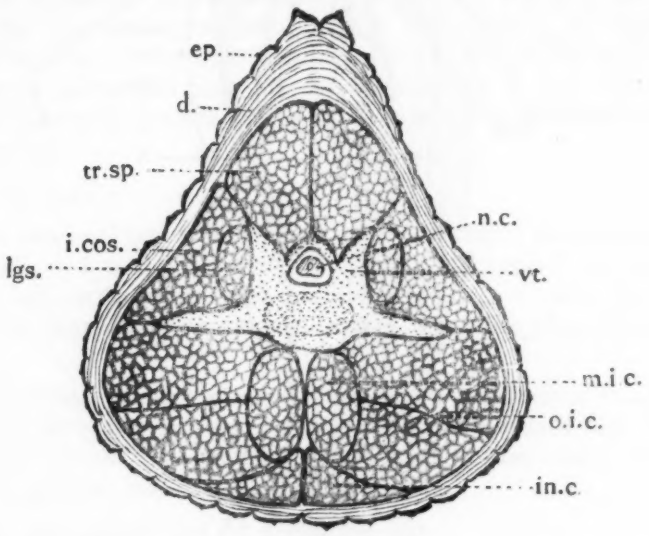

FIG. 5. Transverse section of the tail of *Varanus monitor* passing through the plane Y' of Fig. 7 (× 3). Abbreviations as in previous figures.

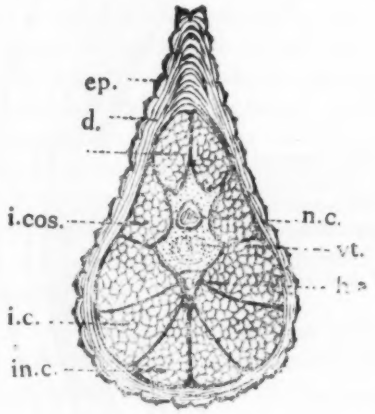

FIG. 6. Transverse section of the tail of *V. monitor* passing through the plane Z of Fig. 7 (× 3). Abbreviations as in previous figures.

zeylanicus and other lizards, where the ilio-costalis muscle is absent in the tail and there are only two pairs of epiaxonic muscles. Both the longissimus and iliocostalis muscles occupy a gutter-shaped depression between the zygapophyses and transverse processes of the anterior caudal vertebræ, but

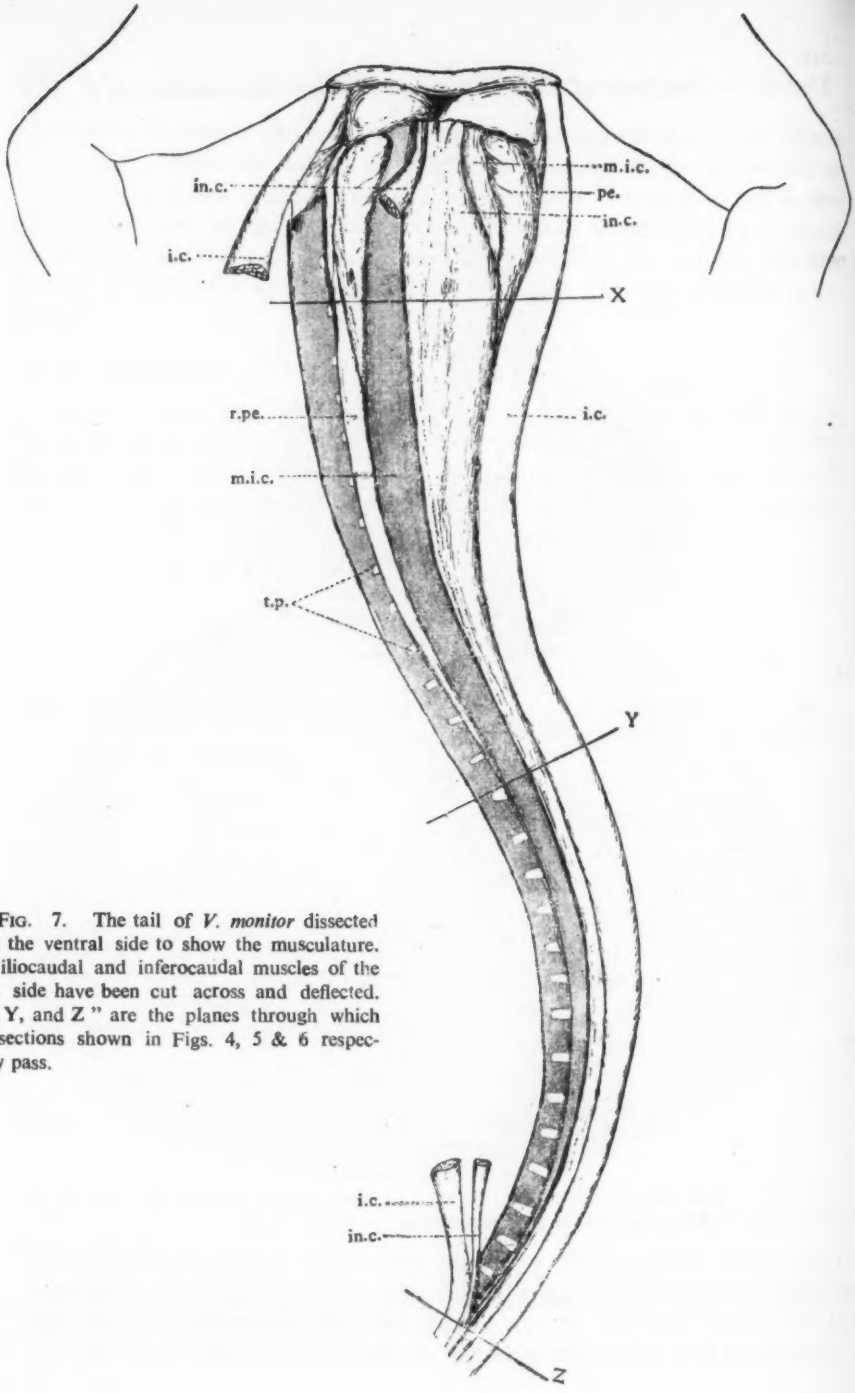

FIG. 7. The tail of *V. monitor* dissected from the ventral side to show the musculature. The iliocaudal and inferocaudal muscles of the right side have been cut across and deflected. " X, Y, and Z " are the planes through which the sections shown in Figs. 4, 5 & 6 respectively pass.

only one of these muscles extends up to the end of the tail. It is clear from a study of successive transverse sections (Figs. 4–6), that it is not the ilio-costalis but the longissimus muscle which disappears in the middle and posterior regions. As we trace the longissimus backwards, we find it becoming laterally compressed and gradually diminishing in size. Its posterior part lies completely covered by the ilio-costalis and it ends almost halfway down the tail.

(3) *M. Ilio-costalis*

This muscle (Figs. 4–7) occupies a lateral position just external to the *longissimus*, and rests partly on the transverse processes of the caudal vertebræ and partly on the tough mesaxonic septum. It covers the posterior part of the longissimus muscle and extends along the whole length of the tail.

The attenuation and disappearance of the longissimus muscle in the middle caudal region and the extension of the ilio-costalis right up to the end of the tail are important features not hitherto described. Their significance is pointed out later on.

(4–6) *M. Ilio-caudalis* and *M. Infero-caudalis*

If we leave the additional musculature occurring in the base of the tail, the hypaxonic set (Figs. 4–7) consists of the ilio-caudalis and the infero-caudalis. The former is divided into two parts, but these (*Pars externalis* and *Pars medialis*), instead of being one dorsal and the other ventral, lie side by side longitudinally in the space beneath the transverse processes and lateral to the hæmal arches. The *Pars externalis* is further subdivided into a dorsal and a ventral portion. The *Pars mesialis* does not extend up to the extremity, but ends at about the middle of the tail. It is separated from its fellow of the other side partly by the hæmal arches and partly by the vertical septum, while the two infero-caudal muscles are separated from each other only by a vertical connective-tissue septum extending downwards from the apex of the hæmal arch.

The *infero-caudalis* is characterised by the presence of fine tendons which run from the mid-ventral groove backwards and disappear into the longitudinal tendinous line on the surface of the muscle.

VII. Discussion

This kind of tail is a powerful organ adapted for vigorous lateral movements of a lashing type. On close observation a series of sinuous contractions are seen to pass alternately on each side from the base of the tail towards the end. The undulations start in the trunk and pass from there

backwards. There is an ostensible similarity in their origin and course of direction to the sinuous movements in the lash of a whip. In order to find out whether these movements could originate in the tail itself, or were waves of contraction induced by those of the trunk, the following experiments were performed:

Experiment 1

A string was tied tightly around the waist of a monitor lizard (*Varanus monitor*) and the creature dropped into a tank of water. The animal, although it seemed to be considerably lacking in balance, managed somehow to keep itself floating with its head outside the surface of water and the tail hanging inside it. When poked with a stick and pushed down under the water surface, it struggled to rise up with awkward movements of the trunk and limbs, but scarcely those of the tail.

This experiment indicates that the helplessness of the lizard, especially its lack of balance, is due to the pressure of the tight ligature on the waist which hinders the undulatory movements of the trunk from passing on freely into the tail. The fact that the trunk musculature is also incapacitated from performing the sinuous movements signifies that the trunk and tail form a single organic system of which the components cannot work properly in isolation. The following experiment was devised to confirm these inferences.

Experiment 2

The lizard of the first experiment was taken out of water and the loop of string relaxed so as to remove as much of the pressure on the waist as possible. On lowering again into water, the animal was found to be quite active this time. It swam, well balanced, with vigorous undulating movements of the trunk and tail while the limbs helped in changing the direction and position of the body.

It is clear, therefore, that the undulations of the tail start not at its base but somewhere in the posterior part of trunk. They pass backwards towards the extremity, and end there almost like the series of wave-like movements in a whip.

On account of such a function, the tail shows a number of interesting features in its structure. It is laterally compressed almost in its entire extent. The skin is tough and closely attached to the underlying muscles. The lateral scales are not imbricate, but juxtaposed. There are numerous spaces between the transverse rows of lateral scales. The vertebræ are compact and strongly built, and are modified in their mode of articulation

for lateral movements. The musculature consists of longitudinal muscle trunks, devoid of myomeric segmentation and clearly differentiated into lateral sets rather than dorsal and ventral ones.

The fact that the ilio-costalis muscle, but not the longissimus, is continued up to the end of the tail and forms an important caudal muscle is a remarkable feature which explains the conduction of the waves of undulations from the trunk into the tail. As already known, the ilio-costalis is responsible in other reptiles for the performance of creeping movements by the trunk, and such movements are similar to those found in *Varanus monitor*.

VIII. Summary

The undulating type, exemplified by *Varanus monitor*, was studied both morphologically and experimentally. It shows the following adaptations for the vigorous side-to-side movements:

(*a*) The tail, although thick and rather dorso-ventrally depressed at its base, presents a lateral compression along the greater part of its length.

(*b*) The epithelium, which is leathery in consistency, is devoid of lines of cleavage and is closely attached to the underlying musculature.

(*c*) For every two rows of ventrals, there are three rows of laterals—an arrangement which provides for stretching.

(*d*) The vertebræ are strongly built to withstand the stress and strain of lashing, and are characterized by the laterally-elongated centra and the relatively small size of the zygapophyses and transverse processes. Near the end of the tail the neural spines and other processes atrophy.

(*e*) The caudal musculature, except in the anterior part of the tail, consists of five pairs of muscle trunks which run longitudinally up to the end of the tail, two being dorsal to the mesaxonic septum and three ventral to it. The longissimus ends before it reaches the middle of the tail and the ilio-costal extends backwards from the trunk right up to the end of the tail. There is no such system of tendons as in *Chameleon*.

IX. Acknowledgments

The present work was carried out under the direction of Professor Beni Charan Mahendra to whom I owe a deep sense of gratitude for his painstaking assistance and able guidance.

X. Bibliography

A comprehensive bibliography has already been given by the author in Parts I and II of the present series (*Proc. Ind. Acad. Sci.*, Vol. XIII, No. 3 and Vol. XXVIII, No. 1), to which reference might be made.

THE EFFECT OF ASPHYXIA ON THE RESPONSE OF UNSTRIATED MUSCLE TO IONS AND DRUGS

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The evidence is conflicting as regards the effect of asphyxia on the response of unstriated muscle to ions and drugs. Lovatt Evans (1919) found that pilocarpine produced powerful contraction of rabbit's gut under anoxæmia produced by oxygen lack or cyanide, but the contraction lasted for three minutes, instead of over 50 minutes with oxygen and the gut responded only once with cyanide. Gross and Clark (1923) observed that the asphyxiated gut did not respond to pilocarpine but responded to potassium and barium. Garry (1928) found that pituitary extract on the guinea pig's uterus and histamine on the rabbit's gut produced no effect in the absence of oxygen. Schmitt and Nicoll (1933) observed that arecoline, pilocarpine and acetylcholine failed to produce contraction of the intestinal strips when they were under the influence of sodium cyanide. Prasad (1936) found that asphyxiated cat's colon responded to acetylcholine in the presence or absence of glucose.

In the present research an attempt has been made to elucidate the effect of asphyxia on the response of unstriated muscle to ions and drugs.

Experimental

The methods used were as described previously (Singh and Singh, 1948). The experiments were performed upon unstriated muscle of frog's stomach, dog's stomach and fowl's gut. For comparison of the effects of asphyxia on the responses to acetylcholine and potassium, concentrations were used that produced contractions of equal tension (about 20 g.).

Results

If the muscle is asphyxiated, it at first becomes hyperexcitable to potassium and acetylcholine. Thereafter the responses decline as with electric current; the rate of decline of excitability depends upon the frequency of stimulation. Thus with alternating current, at 30° C. frog's stomach may become inexcitable in about 15 minutes if stimulated every minute, but may

take over 6 hours if stimulated every 15 minutes. Similarly with potassium and acetylcholine the muscle may respond for several hours if stimulated every 10 or 15 minutes. If the potassium is continuously present, producing tone, then the muscle may become inexcitable in a few minutes.

The decline of the response to potassium and acetylcholine depends upon the kind of muscle used. Thus the decline is rapid with fowl's gut, but slow with dog's or frog's muscle. The reason for this difference appears to be due to the nature of the tone exhibited by the muscle. If the muscle is in lactic tone and showing spontaneous contractions, then the inexcitability is rapid, because energy has to be shared between the tone and the response to potassium or acetylcholine. If the muscle is in alactic tone, then a greater amount of energy will be available for the responses, and so the decline of these will be slow. Thus the inexcitability following asphyxiation depends upon the following factors: (1) the nature of tone, (2) the presence or absence of spontaneous contractions, (3) the frequency of stimulation. If these factors are taken into consideration, then the discrepancy between the results of various observers disappears.

The above conclusions are supported by the following experiment. In dog's stomach and frog's stomach, the asphyxial decrease in excitability is more rapid if the temperature is raised from 30° C. to 35-38°C. Like tone, increase in temperature increases the metabolism.

The effects of sodium cyanide (1 in 10,000) resemble those of asphyxia.

An asphyxiated muscle, stimulated by alternating current in alkaline solutions, becomes inexcitable to the current, but the response to potassium and acetylcholine remains though diminished. This shows that the latter two also derive energy from some other source. If the muscle is stimulated alternately with potassium and acetylcholine, the response to latter disappears first, or if the muscle is stimulated only with acetylcholine, the response to potassium is produced after the muscle has become inexcitable to acetylcholine. This shows that the response to potassium and acetylcholine derive energy from different sources respectively. The response to acetylcholine requires more energy than potassium. This is in accord with the view expressed as regards the mode of action of acetylcholine (Singh, 1944, 1945; Singh and Singh, 1947). The process of sensitisation would be responsibe for the extra energy used by acetylcholine. The resistance to asphyxia, therefore increases in the following order: Nervous stimulation < electrical stimulation < acetylcholine < potassium.

The muscle also becomes inexcitable to pilocarpine before potassium or barium.

Action of tone-producing substances.—It was found that when the muscle became inexcitable to alternating current, tone-producing substances restored the response (Singh and Singh, 1948). In frog's muscle the result was just the opposite, if it was stimulated less frequently, about once in 10 or 15 minutes; as the muscle is usually stimulated as such with potassium or acetylcholine, their responses were also suppressed. The action of these substances varied in the same order as before $K > NH_4 > Li > Na$; $Cl < Br < NO_3 < I < SCN$; $Ca < Sr < Ba$. Eserine (1 in 10^6) suppressed the response to acetylcholine. As the action of acetylcholine is less potent than that of potassium, the latter may suppress its own response, and the asphyxiated muscle then responds to acetylcholine, but not to potassium, a result exactly opposite of that described above (Fig. 1). After the addition of potassium, the muscle ceases to respond to acetylcholine.

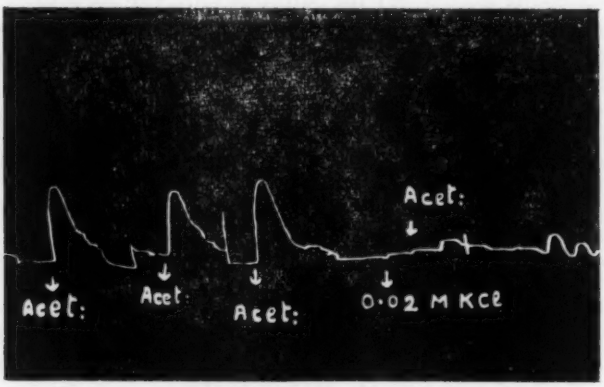

FIG. 1. Frog's stomach muscle, asphyxiated. Stimulation by acetylcholine (1 in 50,000). Potassium 0·02 M KCl, abolishes the response.

The above experiment shows that the metabolism frog's of unstriated muscle differs with activity. When the activity is great, energy is derived from some other source than when it is lowered ; these two sources are antagonistic.

The above action of the tone-producing substances on frog's muscle is variable; in some muscles it is less marked it may also be obtained the presence of oxygen, though the usual response is that of potentiation, as in *Mytilus* muscle (Singh, 1938). In mammalian muscle, there is potentiation instead of suppression, both in the presence and absence of oxygen.

The effects of sodium cyanide are similar to those of asphyxia. The response of frog's muscle to acetylcholine may disappear within a few minutes.

Action of glucose.—In the presence of oxygen, glucose 0·1 per cent. has an inhibitory action on the response to potassium and acetylcholine at 25-30° C. this inhibitory phase is succeeded by one in which the responses are increased (Figs. 2, 3). If the metabolism is increased by raising the temperature, to 35-38° C. or by continuous presence of potassium or if considerable lactic tone is present, then the inhibitory phase may be absent, the response increasing from the outset. These experiments show that the metabolism of unstriated muscle is changed by glucose ; utilisation of glucose appears to be increased by its presence in the saline or by increased metabolism. These results on unstriated muscle are similar to those on the heart muscle. (Best and

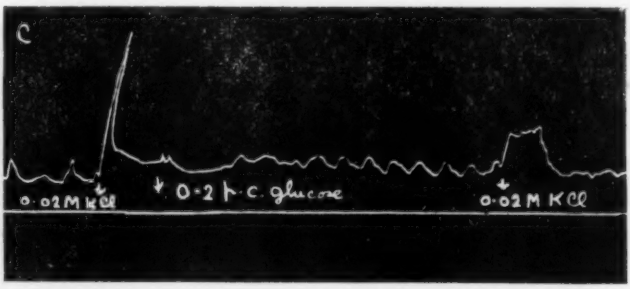

FIG. 2. Frog's stomach muscle, asphyxiated. Stimulated by 0·02 M KCl. Action of 0·2 per cent. glucose.

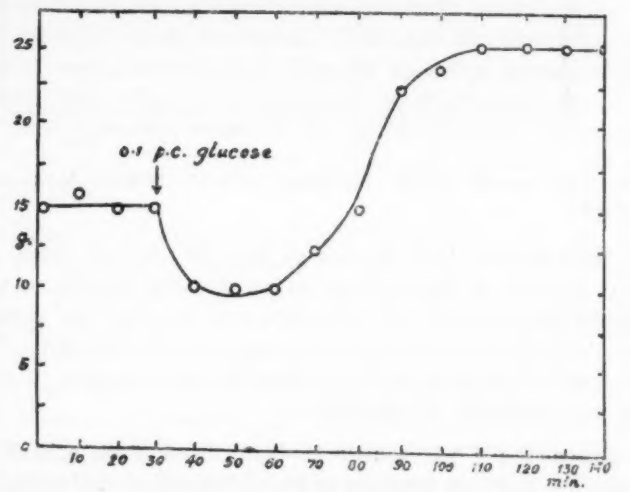

FIG. 3. Frog's stomach muscle. Stimulated by 0·02 M KCl. Action of 0·1 per cent. glucose in the presence of oxygen.

Taylor, 1945). In the heart muscle glycogen appears to be used only as emergency fuel, that is when heavy work is undertaken. Under certain conditions only a small fraction of the oxygen consumed by the heart can be accounted for by the oxidation of carbohydrate but with a high blood sugar, the respiratory quotient may be 0·95 or 1·0 indicating a high carbohydrate utilisation.

The above results can be understood by a study of the action of iodoacetic acid (1 in 10,000). Iodoacetic acid causes either increase or diminution of the response. The diminution may be followed by an increase (Fig. 4). Ultimately the response declines. If iodoacetic acid

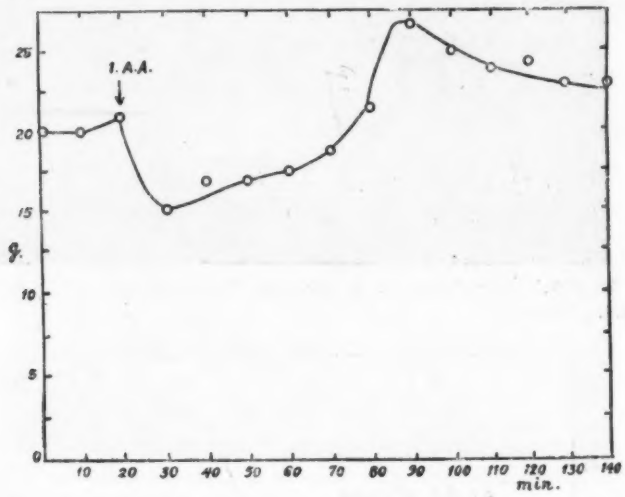

FIG. 4. Dog's stomach muscle. Stimulated by 0·025 M KCl. Action of iodoacetic acid (1 in 20,000).

decreases the response, then the energy must be derived from glycolysis. Subsequent increase of the response shows that the function is taken over by a non-glycolytic source. If iodoacetic acid increases the response from the outset, then this source comes into operation immediately. The action of glucose and iodoacetic acid shows that the above source of energy and glycolysis are mutually antagonistic.

The action of pH 6 is similar to that of iodoacetic acid (Fig. 5). It will be noticed that the response at first decreased in acid solution, and it suddenly increased when the depression had touched the zero point. It appears that up to the zero point the glycolytic source was functioning,

and so long it was doing so, the non-glycolytic source did not come into operation. As soon as the former source ceased functioning the latter source came into operation.

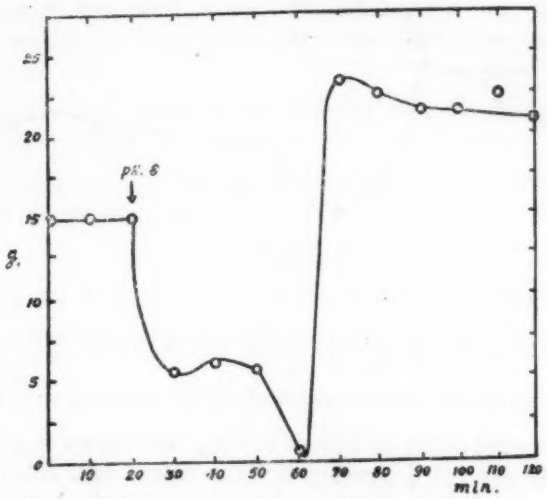

FIG. 5. Frog's stomach muscle. Stimulated by 0·02 M KCl. Effect of pH 6.

The response to alternating current is usually increased by glucose, and iodoacetic acid causes depression. This may be related to the very much greater oxygen consumption when the muscle is stimulated by alternating current than when it is stimulated by potassium, glycolysis then being the chief source of energy. In *Mytilus* muscle, iodoacetic acid abolishes the response to alternating current and increases that to potassium (Singh, 1938).

In the absence of oxygen, at 25°-30° C. glucose has an inhibitory action on the response to potassium (Fig. 6). and acetylcholine, which is followed by partial recovery, glucose being inferior to oxygen in sustaining the response, as happens when the muscle is stimulated with alternating current. This action is produced if the action of potassium is powerful, that is, when the response does not decline considerably or increases in the absence of oxygen. If however, the response declines, indicating exhaustion of the muscle, or if the metabolism is increased by raising the temperature or by continuous presence of potassium, or if the muscle had shown much lactic tone in the presence of oxygen, then glucose has a stimulatory action from the start.

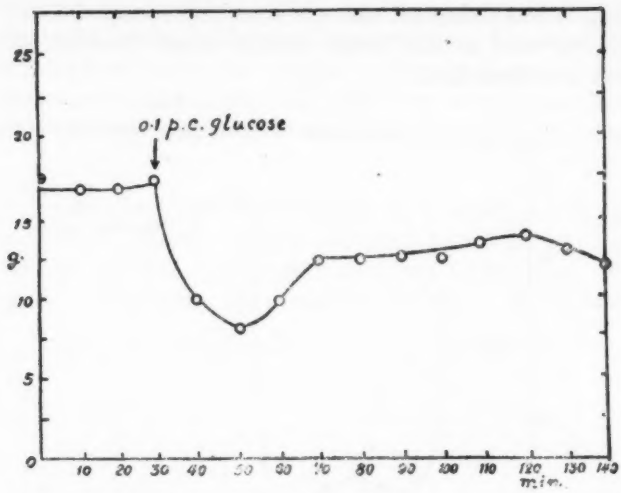

FIG. 6. Frog's stomach muscle, asphyxiated. Effect of 0·1 per cent. glucose.

The depressant effect of tone-producing substances is antagonised by iodoacetic acid and pH 6 showing that the antagonistic effect is produced when the energy of the previous contractions is derived from glycolysis. Glucose thus increases the depressant effect. The presence of glucose changes the metabolism, so that after about 10 minutes, it has a potentiatory effect. If the tone-producing substances potentiate the responses, then the energy must be derived from similar sources.

DISCUSSION

Normally under aerobic conditions, the sources of energy for the response to potassium and alternating current respectively are different. The metabolic mechanisms of tone and twitch contractions are therefore different. When the demand is excessive, they may utilise energy from a common pool. Further it appears that the muscle does not utilise energy simultaneously from two sources; when one source is opened the other is closed.

SUMMARY

1. Asphyxia at first increases the responses to potassium and acetylcholine, then depresses them.

2. The decline of excitability is affected by the nature of tone.

3. The resistance to asphyxia of various responses varies in the following order: Nervous stimulation < electrical stimulation < acetylcholine < potassium.

4. Tone-producing substances depress the response of asphyxiated frog's unstriated muscle, if the latter is stimulated about once in 10-15 minutes. In mammalian muscle the result is opposite.

5. Glucose has at first an inhibitory and then a stimulatory action on the response of unstriated muscle to potassium; if the metabolism is increased by raising the temperature, then the effect becomes stimulatory from the outset.

6. Iodoacetic acid increases the response to potassium after a preliminary depressant effect.

7. pH 6 increases the response to acetylcholine and potassium after a preliminary depressant effect.

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STUDIES IN CONTACT TOXICITY

Part II. Reactivation of Surface Treated with Dichloro Diphenyl Trichloroethane (DDT) by Storage in a Burma Teak Box

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Introduction

In Part I of this series of investigations it was shown that successive generations of a laboratory bred culture of *Culex fatigans* Wied showed increasing resistance to contact insecticides like DDT. In the present paper an account is given of a second distinct effect which was observed during the main evaluation work on contact insecticides. This relates to a small Burma teak box in which the glass plates sprayed with DDT were generally stored between exposure to mosquitoes. The result of storage of plates with feeble activity in this box is to raise their activity, very considerably. This effect, although it is temporary in nature can, nevertheless, be repeated everytime the plates are stored inside the box. Careful investigation has shown that the reactivation is confined to Burma teak box only. Other timbers like Mango (*Mangifera indica*, Linn.), Pine (*Pinus longifolia* Roxb.) and *Shisham* (*Dalbergia sissoo* Roxb.) do not show the effect. What is more surprising is the fact that even teak from Central Provinces (India) shows difference in behaviour from Burma teak. The conditions under which the effect can be nullified have been found although the exact mechanism of the effect has not been understood. Probably the latter is connected with the (invisible) deposit of some emanation from the walls of the box on the test surfaces during storage. This aspect is under investigation and will be reported in a future communication.

Materials

1. *Burma Teak Box.*—This was made from planks $\frac{1}{2}''$ thick and was of dimensions $12'' \times 10'' \times 4''$. The box was provided with a lid working on hinges (Fig. 1) and had grooves inside to accommodate two rows of glass plates $4'' \times 3''$. Boxes of the similar description but made of Mango, Pine, *Shisham* and C.P. teak were available for comparison. The boxes were used in the unpolished condition.

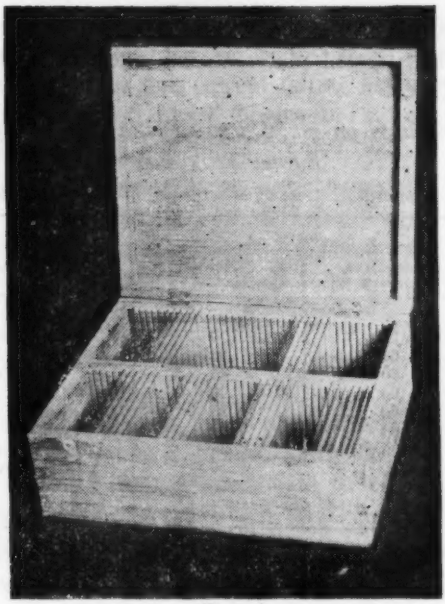

FIG. 1

2. *Test Insects.—Culex fatigans* adults, 36 hours old, bred under standard conditions as described earlier[1] were used as test insects. Twenty insects were used in each test.

3. *Testing Technique.*—This was carried out according to the method described previously[1] using a box composed of six glass plates sprayed with the insecticide. The experiments reported in this paper were carried out in the same room where the insects were bred, at 85 ± 1° F. and 72 ± 3% R.H.

4. *Insecticide.*—The insecticide used was Dichloro Diphenyl Trichloroethane, DDT, and was sprayed as a 5% emulsion on glass plates. The emulsion was derived from the following miscible oil concentrate:—

DDT	..	25	parts by weight (pp' isomer 65%)
Toluene	..	32·4	,,
Turpentine	..	32·4	,,
Alcohol (absolute)	..	5·3	,,
Soap powder	..	3·4	,,
Distilled water	..	1·5	,,

Results

1. *The effect.*—Table I below summarises the results of exposures carried out on DDT treated plates stored alternately on the (laboratory) bench and in the box.

TABLE I. *Exposure tests on plates treated with DDT. Effect of storage in Burma Teak Box*

(DDT concentration 35 mgm./sq. ft. Date of treatment by mist deposition technique, 19–12–1495.)

Age of deposit in days	Contact period in minutes	Percentage* mortality	Storage conditions in between exposures
1	20	100	Initial exposure
15	60	50	Assembled on the bench
19	60	35	do
24	60	100	In the box
30	60	30	Assembled on the bench
40	60	75	In the box
47	60	35	Assembled on the bench
62	60	0	do
70	60	55	In the box

* Mortality in control in all cases was zero.

The striking enhancement in the mortality rate of insects when exposure is carried out on plates after their storage in the Burma teak box, will be

TABLE II. *Exposure tests on plates treated with DDT and stored under different conditions*

Set A.—Stored in the Burma teak box.
Set B.—Stored (assembled) on the (Laboratory) bench.
Set C.—Stored (assembled) on the (Laboratory) bench during the day and in the box during the night.

(DDT concentration in all plates: 51 mgm./sq. ft. Date of treatment, 8–4–1946.)

Age of deposit in days	Contact period in minutes	Percentage Mortality*			Remarks
		Set A	Set B	Set C	
4	20	20	20	20	Initial exposure before storage
9	20	40	15	30	First exposure after storage
24	40	100	45	90	Contact period was increased to 40 mts. in order to bring into bold relief the differences in mortality rates
45	40 for A & C 60 for B	55	50	50	..
84	60	90	15	70	..
116	60	60	0	10	..

* Mortality in control in all cases was zero.

noted. In order to confirm this observation, three further sets of plates were prepared. Four days after treatment with DDT, one set was stored on the bench assembled throughout the period of experiment (4 months). The second was stored in the box (except during exposures) and the third was left assembled on the bench during the day and stored in the box during the night. The results of this experiment are described in Table II.

The results in Table II again emphasise the peculiar effect of storage of surfaces treated with DDT in the box on their activity. The plates stored alternately outside and inside the box (Set C) show activity intermediate between those stored continuously inside (Set A) and outside (Set B).

2. *Assesment of likely causative factors:*

(*a*) *Light and Darkness*: *Humidity.*—In attempting to investigate the mechanism of the effect described above, it was considered of interest to study whether the effect was related to the physical state of light or darkness. In other words, any reactivating influence which darkness may have on the insecticidal surfaces during storage was sought to be investigated. In this connection three sets of plates treated with DDT under identical conditions were stored as follows between exposures:—

Set A.—In a glass desiccator over a saturated solution of NaCl (75% R.H.). The desiccator was suitably wrapped on the outside to shut off light completely.

Set B.—As in Set A, but the desiccator was left in light.

Set C.—In the (Burma Teak) box.

The results of exposure tests are given in Table III.

TABLE III. *Influence of light/darkness during storage on the activity of insecticidal surfaces*

(DDT concentration in all plates : 45 mgm./sq. ft.)

Age of deposit in days	Contact period in minutes	Percentage Mortality*			Remarks
		Set A	Set B	Set C	
1	30	55	40	45	Before storage
2	30	55	40	55	First exposure after storage
4	30	30	25	90	Second exposure after storage
6	30	10	10	100	Third exposure after storage
7	30	10	10	90	Fourth exposure after storage

* Mortality in control in all cases was zero.

The above results while revealing again the characteristic effect of storage in the box, show that light or darkness cannot be a contributing cause.

The measurement of temperature inside the box during storage of the plates showed that it was identical with that of the surroundings and this would indicate that the humidity also remained at the same level as that outside. It would appear, therefore, that temperature and humidity *per se* are not factors associated with the phenomenon of reactivation.

(*b*) *Nature of timber of which the storage box is made.*—At this stage it was decided to investigate whether boxes made of timbers other than Burma Teak could also reactivate surfaces treated with DDT when the latter are stored in them. Boxes of identical dimensions made of Mango, Pine, *Shisham* and C.P. teak were tested side by side with the Burma teak box. The results are described in Tables IV, V and VI below:—

TABLE IV. *Burma teak box against Mango and* Shisham *boxes*

(DDT concentration on plate : 55 mgm./sq. ft.)
Set A.—Stored in Burma teak box.
Set B.—Stored in Mango wood box.
Set C.—Stored in *Shisham* box.

Age of deposit in days	Contact period in minutes	Percentage Mortality*			Remarks
		Set A	Set B	Set C	
2	30	25	20	20	Initial exposure before storage
4	30	100	15	10	First exposure after storage
5	30	85	15	15	Second exposure after storage

* Mortality in control in all cases was zero.

TABLE V. *Burma teak box against C.P. teak box*

(DDT concentration on plates : 55 mgm.-sq./ft.)
Set A.—Stored in Burma teak box.
Set B.—Stored in C.P. teak box.

Age of deposit in days	Contact period in minutes	Percentage Mortality*		Remarks
		Set A	Set B	
2	30	10	10	Initial exposure before storage
4	40	65	0	First exposure after storage
5	40	55	0	Second exposure after storage
10	40	55	0	Third exposure after storage
21	40	35	0	Fourth exposure after storage

* Mortality in control in all cases was zero.

TABLE VI. *Burma teak box, pine wood box and storage in the assembled state on the bench*

(DDT concentration on plates 58 mgm./sq. ft.)

Set A.—On the bench.

Set B.—In Burma teak box.

Set C.—In Pine wood box.

Age of deposit in days	Contact period in minutes	Percentage Mortality*			Remarks
		Set A	Set B	Set C	
1	30	45	60	35	Initial exposure before storage in boxes
7	60	35	25	40	do
9	60	30	70	40	First exposure after storage
15	60	10	85	20	Second exposure after storage
19	60	5	80	10	Third exposure after storage
24	60	5	20	90	After the last exposure the storage of Sets B and C was reversed
28	60	0	5	80	..
33	60	0	0	70	..
38	60	0	0	75	..

* Mortality in control in all cases was zero.

Tables IV, V and VI show that the reactivating effect is confined to Burma teak box only, and that boxes made of other timbers including C.P. teak do not activate the insecticidal surface. Further, an examination of Table VI would reveal the fact that Sets B and C (stored in Burma teak and Pine wood boxes), both 19 days old, gave 80% and 10% kills respectively. When after this period the storage of these two sets was reversed, the exposure on the 24th day showed that the erstwhile set C gave 90% kill while the activity of the hitherto potent Set B had fallen, leading to only 20% kill.

It remained to investigate whether the effect was shown only by the particular Burma teak box described hitherto and not by any other box made of Burma teak. For this purpose a second box was made of this timber and was examined with respect to the activation of plates on storage. Table VIII describes the results:

Table VII. *Results with Burma teak box No.* 2

(DDT concentration on plates : 39 mgm./sq. ft.)
Set A.—Stored in new box.
Set B.—Left assembled on bench.

Age of deposit in days	Contact period in minutes	Percentage Mortality*		Remarks
		Set A	Set B	
1	40	30	35	Initial exposure before storage
2	40	80	30	First exposure after storage
4	40	80	25	Second exposure after storage
10	40	25	0	Third exposure after storage
13	40	60	20	Fourth exposure after storage

* Mortality in control in all cases was zero.

From the above it is clear that the effect is specific for Burma teak and is not restricted to any particular box made of this timber.

(c) *Influence of passage of air through the Burma teak box when the plates are in storage.*—It was considered of interest to investigate any possible suppression of the effect as a result of passing air through the Burma teak box with the DDT treated plates in storage. For this purpose, two sets of glass plates were prepared. One set (A) was stored in one of the two compartments of the box (see Fig. 1). For the purpose of the present experiment, this particular compartment was provided with inlet and exit holes. The other set (B) was stored in the remaining compartment and it was ensured that with the lid of the box closed, this compartment was airtight.

TABLE VIII. *Aspiration of air through the Burma Teak box during storage of plates.*

(DDT concentration : 37 mgm./sq. ft.)
Set A.—In the aerated compartment.
Set B.—In the closed compartment.

Age of deposit in days	Contact period in minutes	Percentage Mortality*		Remarks
		Set A	Set B	
1	40	30	30	Initial exposure before storage
3	40	35	100	As per conditions of storage described above
6	40	20	90	do
8	40	60	20	Position of Sets A and B reversed
10	40	85	0	..

* Mortality in control in all cases was zero.

A current of air (at 75% R.H.) was drawn through the compartment holding Set A for a period of 48 hours. The plates were then removed and exposed to the insects. After the exposure, the plates were put back and the aeration continued for a further period of 72 hours and then re-examined. At this stage the position of Sets A and B in the box was reversed and the experiments continued. The results are described in Table VIII.

The results in Table VIII show that whatever reactivation the Burma teak box can bring about, it can be nullified by passing air through the box.

(*d*) *Storage of plates sprayed with emulsion* minus *DDT in the Burma teak box.*—A normal set of plates sprayed with DDT emulsion was tested against a second set which had been sprayed with the emulsion *minus* DDT. Both the sets were stored in the Burma teak box. The results are given in Table IX.

TABLE IX. *Plates sprayed with DDT against those sprayed with the emulsion not containing DDT*

(DDT concentration : 41 mgm./sq. ft.)
Set A.—DDT.
Set B.—Control (Emulsion minus DDT).

Age of deposit in days	Contact period in minutes	Percentage Mortality*		Percentage Knock down	
		Set A	Set B	Set A	Set B
1	30	10	0	75	0
5	40	65	0	95	40
10	40	55	0	90	20

* Mortality in controls in all cases was zero.

Table IX shows that the effect of storage in the Burma teak box is one of reactivation of insecticidal surface only and that no transfer of any insecticidal material from the box takes place. Nevertheless, the high 'knock down' achieved by the Set B is significant.

CONCLUSIONS

The results described above show that glass plates sprayed with DDT and possessing feeble activity against *Culex fatigans* have their activity enhanced in a striking manner as a result of their storage in a box made of Burma teak for a period of 48 hours and longer. This reactivation is of a temporary nature but can be produced again and again by storage in this box. It has been shown that the property of reactivation is specific to Burma teak and is not possessed by Mango wood, Pine wood, *Shisham* and

C.P. teak. Light, darkness, humidity or temperature would not appear to be related to the phenomenon but the latter can be completely negatived by aeration of the box during the storage of the plates inside. A further systematic examination of teak wood is expected to throw light on the mechanism of reactivation, and this is in progress.

Summary

Experiments are described which show that glass surfaces treated with DDT exhibiting feeble activity against *Culex fatigans* Wied can be reactivated as a result of their storage in a box made of Burma teak. The reactivation appears to be specific for Burma teak, and results obtained with other timbers are described. Some experiments have been carried to elucidate the mechanism of reactivation. Further work is in progress.

Acknowledgement

The authors' thanks are due to Dr. T. S. Subramanian for his interest in the work and to the Deputy Chief of General Staff (Weapons and Equipment) and the Director of Technical Development, Army Headquarters, New Delhi, for kind permission to publish the paper.

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138	4	Quadratic curve of the second degree	Quadratic curve
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139	9	4·33189	− 4·33189
152	4	the growth at any stage	the growth rate at any stage
152	10	I the lower asymptote, K the upper asymptote, *e* the Napierian logarithm	I the lower level K + I the upper level, *e* the base of the Napierian logarithm

EFFECT OF ACENAPHTHENE ON YEAST STRAINS OF DIFFERENT GENIC AND CHROMOSOMAL CONSTITUTIONS

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Introduction

Do yeast strains of differing genic and chromosomal constitutions react in an identical fashion when treated with acenaphthene and colchicine? A rational explanation of the contradictory results obtained by different investigators would be possible if it could be shown that strains of varying genic and chromosomal constitutions react differently when treated with the above chemicals. Richards (1938) found an acceleration in the rate of growth when the yeast strain used by him was grown in media containing colchicine. Levan and Sandwall (1943) on the other hand, could not find any such stimulation. The results recorded by Richards and Levan and Sandwall are based on experiments with different strains of yeasts. Their results are not strictly comparable and offer no evidence for any generalization. It is known that the genotype as a whole and occasionally even a specific gene (Rhoades, 1938; Dobzhansky, 1947) may control the mutability of one or more of the genes. The chromosomal constitution of the strains appears to be a much more important factor. While autopolyploidy could be induced in a diploid with known chemical and physical agencies, these may have no effect at all on a tetraploid or a higher polyploid.

A Critical Evaluation of Some Recent Publications

During the past three years a brewery yeast strain, BY 1, has been under constant observation and most of our experiments on induction of polyploidy by diverse agencies have been carried out on this strain. The intensive study of a few selected strains has enabled us to evaluate critically not only the methods of approach, but also the significance of the divergent results obtained by workers investigating the effect of various polyploidizing agencies on yeasts.

Neither Richards (1938) nor Levan and Sandwall (1943) knew either the chromosome or the probable genic constitution of the strains they were experimenting on. From a study of the growth curves of the diploid and tetraploid it was demonstrated (Prema Bai and Subramaniam, 1947) that Richards' results are capable of a different interpretation. The similarity of the growth curve of our tetraploid, BY 3, to that of Richards' strain in media containing colchicine, led us to suggest that colchicine had in fact induced polyploidy.

Levan and Sandwall (1943) do not appear to have made a distinction between vegetatively growing cells and fermenting ones. The difference between these two is fundamental (Subramaniam, 1947 *b*) as fermenting cells appear to have no genetical future at all. While Richards used actively growing cells for his experiments, Levan and Sandwall (1943) used a culture " which was in lively fermentation " (p. 165). While the majority of the cells were capable of active vegetative multiplication in Richards' experiments, only a very small percentage of cells were capable of doing so in Levan and Sandwall's experiments. Fermenting cultures contain only a very small percentage of cells capable of active vegetative multiplication, and the " rather considerable differences " observed by them between the different *controls* cannot therefore be due to " slight differences in treatment " as suggested by them, but is in all probability the result of different numbers of vegetative cells contained in the same quantity of inocula. In their *controls* for colchicine and acenaphthene series they introduced 280 cells per $mm.^3$ At the end of 20 hours while the former contained 16,125 cells per $mm.^3$ the latter had only 7,870 in the same volume. The fact that the same number of cells were introduced in each case, *viz.*, 280 per $mm.^3$ has little significance since the number of viable cells in the inocula may have been different. If we assume that the inoculum introduced into the *control* for the acenaphthene series contained only half the number of viable cells as that for the colchicine series then, the different cell numbers which they observed, at the end of 20 hours in the *controls* of the two series, appear

probable. Since the percentage of viable cells in a fermenting culture is very small, the same aliquot from a well shaken liquid culture need not contain the same number of viable cells.

The number of cells capable of active vegetative multiplication introduced at the start of the experiment determines the yeast crop at the end of 20 hours. When wide variations in the yeast crop in the *controls* for the different series themselves were observed by Levan and Sandwall (1943) *any evaluation of the actual effect of the various drugs, by estimating the number of yeast cells formed at the end of* 20 *and* 48 *hours, is bound to be inaccurate.*

There appears to be also no justification for their conclusion that " neither colchicine nor acenaphthene, which both have a very pronounced *c*-mitotic effect on higher plants have any such effect in yeast, at any rate not by inducing *c*-mitoses ". Even if we assume that all cells in the inocula used by them were capable of active vegetative multiplication, their claim that " during the first 20 hours the cell number is augmented from some hundred cells to about 10,000 cells per mm.^{3}" implies about seven cycles of multiplication by all the cells. Therefore, the time for each division is about three hours. *Any lag due to the presence of the chemical in the medium should be apparent at the beginning of the experiment. Levan and Sandwall do not appear to have made any cell counts during this crucial period.* Our experience indicates (Subramaniam, 1947) that after a single duplication of the chromosomes, acenaphthene has no further effect on the cells. While our control, BY 1, takes 21 hours to reach the end of the logarithmic phase, the auto-tetraploid, BY 3, takes only 16 hours. Therefore, even if acenaphthene or colchicine had induced a doubling of the chromosomes in Levan and Sandwall's (1943) experiments, the prolongation of the lag phase due to inhibition of cell division would be compensated by the shortened generation time of the new strain. Cell counts made at the end of 20 and 48 hours would give no indication of the changes in the population which had taken place in such cultures. There is also no evidence in published literature that the inhibition of cell division by colchicine and acenaphthene extends for such long periods during which the cells are capable of dividing several times, under normal conditions.

Most of the investigators do not seem to have envisaged the possibility that experiments on the rate of growth in media containing various chemicals will not give a correct picture of the effect. Such media form abnormal environments for yeasts, and if the chemicals induce mutations, the culture at different intervals would contain different numbers of mutants,

depending on (1) the rate of induction of mutations by the drug, (2) the viability, and (3) the rate of growth of the different mutants themselves. Before starting any quantitative experiments proof has to be adduced that the chemical employed does not produce gene or chromosomal mutations. This Levan and Sandwall (1943) have not done. There is no proof that the yeast crop obtained by them are not mixtures of gene and chromosomal mutants.

Material and Methods

A seasonal variation was observed in the characteristics of the giant colonies of our control brewery strain, BY 1, and basing our conclusions on spontaneous (Subramaniam, Ranganathan and Krishna Murthy, 1948) and induced (Subramaniam and Krishna Murthy, 1948) reverse mutations, it was suggested that multiple alleles exist at the locus determining the nature of sculpturing of the colony. The discovery that changes in chromosome constitution do produce changes in the characteristics of the giant colonies (Subramaniam and Ranganathan, 1948) has provided a simple technique for the identification of a tetraploid. In 1945 it was the strain giving giant colonies of the *Smooth* type which was treated with acenaphthene. The procedure employed in making giant colonies has already been described elsewhere (Subramaniam and Ranganathan, 1948). Since during particular months both the *Smooth* and *Rough* types are available in our cultures, actively growing cells of both were again treated for 45 days with acenaphthene. There is also in our collection a strain of distillery yeast which has been shown to be a naturally occurring auto-tetraploid (Ranganathan and Subramaniam, 1948). Active cultures of the distillery yeast were also treated continuously for a year with acenaphthene. At the end of the treatment, the cultures were grown for 24 hours in wort, plated and the various types isolated. Giant colonies of the above were grown for a detailed study of the changes induced.

Observations

In 1945 when the *Smooth* type (Photo 1) was treated for 90 days with acenaphthene, the resultant culture contained two distinct types of cells. These were shown to be a "top yeast" (Photo 3) (Subramaniam and Ranganathan, 1946) and a tetraploid (Photo 2) (Subramaniam, 1945). Since higher polyploids did not appear even after treatment for a year with acenaphthene (Subramaniam, 1947) it was assumed that acenaphthene ceases to have any effect once a chromosome doubling had occurred. Periodically tetraploid sectors could be observed in the colonies of the control. Such *normally occurring tetraploid sectors in Rough I* and *Smooth I* types of colonies

are illustrated in Photos 5 and 4. When the identical *Smooth I* type of cells (Photo 6) were again treated in 1947 with acenaphthene for 45 days, only tetraploids were obtained (Photo 8). The treatment did not produce top yeasts. Since mutations at the locus determining the nature of sculpturing of the giant colony have been demonstrated, it is only reasonable to conclude that mutations at other loci should have also occurred. The production of top yeasts on treatment of the control with acenaphthene appears, therefore, to be conditioned by particular genic constitutions.

A classification of the various types of colonies observed in the control has been given elsewhere (Subramaniam, Ranganathan and Krishna Murthy, 1948). It was assumed that the *Smooth I* type of cells were homozygous for the *Smooth* gene, while the *Rough I* type was heterozygous for the *Rough* allele. The *Rough I* type of cells (Photo 5) because of their different genic constitution were also treated for 45 days with acenaphthene. Only a tetraploid could be isolated after treatment (Photo 9). The giant colony of this tetraploid was slightly different from that obtained from the *Smooth I* type in that it had faint striations radiating to the margin. Such a change in the characteristics of the tetraploid, BY 3, was observed (Photos 7, 8 and 9) during the summer of 1947, when the control was giving *Rough I* type of colonies. In January 1948, the colony of the tetraploid did not show the faint radial striations. Just like the reversibility of the *Rough* to *Smooth*, there appears to be a reversibility in the characteristics of the tetraploids also. But the changes in the characteristics of the tetraploid colony are so insignificant that they may be missed unless looked for.

The giant colony of the tetraploid distillery yeast (Photo 10) is indistinguishable in appearance from that of the tetraploid brewery yeast (Photos 7, 8 and 9). On treatment for one year with acenaphthene it also gave rise to a top yeast and a bottom one. The giant colony of the top yeast (Photo 12) had an appearance similar to that of the top yeast obtained from the brewery strain (Photo 3). But whether they have comparable chromosome constitutions could only be judged after careful cytological investigations. The giant colonies of the distillery yeast before and after treatment (Photos 10 and 11) were indistinguishable in appearance. If tetraploids and octoploids give rise to giant colonies having identical appearance, only cytological investigations would indicate the exact nature of the change that has resulted from such long treatment with acenaphthene.

Discussion

As would be evident tetraploids could be obtained from the two chromosome brewery yeast strains of different genic constitutions by long treatment

with acenaphthene. On the other hand, the characteristics of the giant colonies of the control tetraploid distillery yeast as well as one of the strains isolated after one year's treatment of the above with acenaphthene are identical in appearance. At first sight it would appear that since both the treated and control colonies of the distillery yeast are similar in appearance, even one year's treatment has produced no change at all. The appearance of a top yeast on treatment of the tetraploid definitely indicates that acenaphthene has some action and hence any assertion that the bottom strain isolated after treatment is identical with the control should be substantiated by proof that the giant colonies of the tetraploid and octoploid are different in appearance. Since the cytology of the new strains has yet to be investigated, no conclusions could be drawn from the mere similarity in appearance of the control and treated strains. The above facts should dissuade workers from making hasty generalizations based on meagre data.

Our identification of the tetraploid was based purely on chromosome number. Further studies on the diploid and tetraploid showed (1) that their giant colony characteristics (Subramaniam and Ranganathan, 1948), (2) growth curves (Prema Bai and Subramaniam, 1947), (3) rate of fermentation (Mitra, 1948), and (4) nicotinic acid content (Duraiswami and Subramaniam, 1948) are entirely different. The tetraploid had a shorter generation time and showed only a single growth cycle and an accelerated rate of fermentation. It has also been demonstrated (Prema Bai Mallya, unpublished) that tetraploids produced by treatment of the control with chemical as well as physical agencies not only gave giant colonies of identical appearance, but had also similar growth curves. Thus as far as our control brewery yeast, BY 1, is concerned it is a simple matter to identify a tetraploid merely from the characteristics of its giant colonies.

It is surprising that most of the investigators on yeast (Dodge, Dodge and Johnson, 1941; Levan and Sandwall, 1943; and Richards, 1938) do not seem to have appreciated the importance of isolating stable types after various treatments by simple plating and then comparing their behaviour with that of the control in normal media. So long as qualitative investigations do not precede quantitative ones, much importance cannot be attached to the latter.

We have observed differences in viability and rate of growth of strains assumed to be homo- and heterozygous for a single allele (Prema Bai Mallya and Subramaniam, 1948) and since even in our control cultures grown at room temperature different gene mutants get established during the different seasons of the year, the legitimate conclusion is that even the same

mutant may have different growth rates during the varying seasons of the year. It is natural, therefore, to conclude that media containing specific chemicals may even form (Braun, 1947) selective environments for particular mutants quite apart from the mutagenic action exerted by the chemical itself on the cells growing in such media. Any investigation on the effect of various mutagenic agencies on yeasts should take into consideration the interaction of the host of factors involved for arriving at any generalization.

Quite apart from all this, the claim of Levan and Sandwall (1943) that both colchicine and acenaphthene have no *c*-mitotic effect on yeast is unwarranted as they did not investigate either the cytology of the control or of the cells at different stages of treatment with the drugs.

The tetraploids isolated after treatment of both *Rough I* and *Smooth I* types of the control give rise to giant colonies having a smooth surface and a faintly wavy margin. The faint radial striations observed in the colony of the tetraploid derived from the *Rough I* are so insignificant that they do not necessitate any consideration at all. If the nature of sculpturing of the colony is *gene* determined, the cumulative action of the duplicated *genes* should find an expression in the tetraploid. However, duplication of the *genes*, as happens in tetraploids, actually results in a loss of sculpturing. This result would not strike one as strange when it is remembered that the giant colony is not strictly comparable to tissues of higher organisms.

The doubling of the chromosomes alters usually the surface-volume and nuclear-cytoplasmic ratios and hence should affect the physiology of the cell as a whole. The major changes in the functioning of the cell caused by tetraploidy have to be taken into consideration when trying to evaluate the possible cumulative action of particular genes. The diploid and tetraploid yeasts in our collection differ from each other in cell shape and mode of budding, which appear to be two of the important factors determining the nature of sculpturing of the giant colony. The absence of any type of sculpturing in the tetraploid colony does not, under the circumstances, appear surprising.

The significance of auto- and allopolyploidy in yeasts has been completely ignored by Lindegren (Lindegren and Lindegren, 1946) and Spiegelman (1946) in evaluating the " irregular " genetic behaviour of yeasts observed by them under particular environmental conditions. Their speculations seem to have very little experimental justification. A quotation from Lindegren and Lindegren (1946) given below may justify the above criticism. " We had previously reported that a recessive could be transformed into a fermenter if the recessive spore was cut out of a heterozygous cytoplasm

in the continued presence of the respective substrate. 'Masked' recessives, in which the character was induced by this technique of direct transfer of cytoplasm, could be unmasked by dissimilation in the absence of the respective substrate. *We recently made several attempts to duplicate this experiment, but were not able to produce more 'masked' recessives by continued exposure to substrate than were found in the untreated controls.** This does not necessarily invalidate our first experiment, but indicates at least, that the conditions which control the phenomenon have not been completely worked out" (p. 123). It is on such insufficient data that Lindegren tries to modify the fundamental Mendelian principle of independent assortment and speaks about "recessives acquiring the dominant character at meiosis .....by direct transfer from dominant chromogene to recessive chromogene" (p. 123). In tetraploids incomplete dominance is a common phenomenon owing to the number of genotypes possible. Little (1945) states: "On account of striking differences between the genetics of diploids and tetraploids, plant breeding procedures applicable to diploids must frequently be modified in dealing with tetraploids" (p. 82). While the probability of obtaining a triple recessive in a diploid is 1 in 64, in an auto-tetra ploid, it may range anywhere between 1 in 8,000 to 1 in 46,656, depending upon the rate of incidence of 'double reduction'.

The "Cytogene" and "Plasmagene" concepts would be valid only if it is proved that Lindegren and Spiegelman are not dealing with polyploid segregation.

Summary

1. The effect of acenaphthene on yeast strains of different genic and chromosomal constitutions was investigated.

2. A critical evaluation of the methods of approach as well as the significance of the divergent results obtained by workers investigating the effect of various polyploidizing agencies on yeast is given.

3. In 1945, when the *Smooth I* type was treated for 90 days with acenaphthene, the resultant culture contained a top yeast and a tetraploid. When the identical *Smooth I* type was again treated in 1947 with acenaphthene for 45 days, only tetraploids were obtained. Since mutations at the locus determining the nature of sculpturing of the colony have been demonstrated it is only reasonable to conclude that mutations at other loci should also have occurred. The production of top yeasts on treatment with acenaphthene appear, therefore, to be conditioned by particular genic constitutions.

* Italics are ours.

4. Normally occurring tetraploid sectors in *Rough I* and *Smooth I* types of colonies are illustrated. The *Rough I* type of cells, which have a different genic constitution also gave rise to a tetraploid.

5. A tetraploid distillery yeast when treated for one year gave rise to a top yeast and a bottom one. The giant colonies of the control, and the bottom strain isolated after treatment, were indistinguishable in appearance. Entire reliance should not be placed on mere similarity in appearance of giant colonies and the invalidity of hasty generalizations based on meagre data is emphasized.

6. The absence of sculpturing in the tetraploid colony is not surprising since doubling of the chromosomes alters usually the surface-volume and nuclear-cytoplasmic ratios and hence should affect the physiology of the cell as a whole.

7. The " Cytogene " and " Plasmagene " concepts would be valid only if it is proved that Lindegren and Spiegelman are not dealing with polyploid segregation.

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Description of Photographs

Photo 1. BY 1. Diploid control. 2·4 cm. 12-day growth. 27–9–1946.

Photo 2. BY 3. Tetraploid. 2·1 cm. 12-day growth. 27–9–1946.

Photo 3. BY 2. Top Yeast. 2·2 cm. 12-day growth. 27–9–1946.

Photo 4. BY 1. Diploid control grown in Ragi malt agar. 2·4 cm., 20-day growth. 5–3–1948.

Photo 5. BY 1. Diploid control. 2·5 cm, 10-day growth. 30–9–1947.

Photo 6. BY 1. Diploid Smooth-Reverse Mutant. 3·2 cm., 25-day growth. 14-11-1947.

Photo 7. BY 3. Tetraploid. 14-day growth. 27–6–1947.

Photo 8. BY 3. Tetraploid. 2·3 cm., 9-day growth. 28–7–1948.

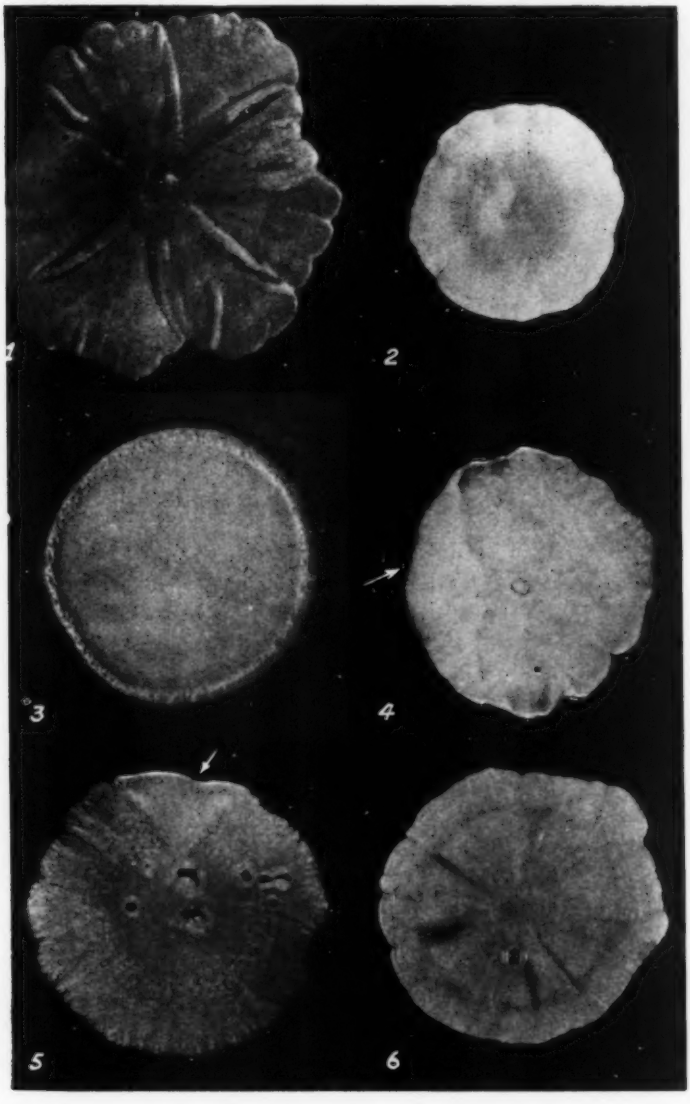
Photo 9. Tetraploid obtained by treating the *Rough 1* type of cells. 2·7 cm., 13-day growth. 3–11–1947.

Photo. 10. DY Tetraploid-control. 2·7 cm., 15-day growth. 10–4–1947.

Photo. 11. DY Bottom Yeast obtained after one year's treatment with acenaphthene. 3·0 cm., 23-day growth. 10–4–1947.

Photo. 12. DY Top Yeast obtained after one year's treatment. 2·9 cm., 31-day growth 21-4-1947.

Except in the case of Photo 4 all the colonies were grown in Barley-Malt-agar. The measurement of the longest diameter is given.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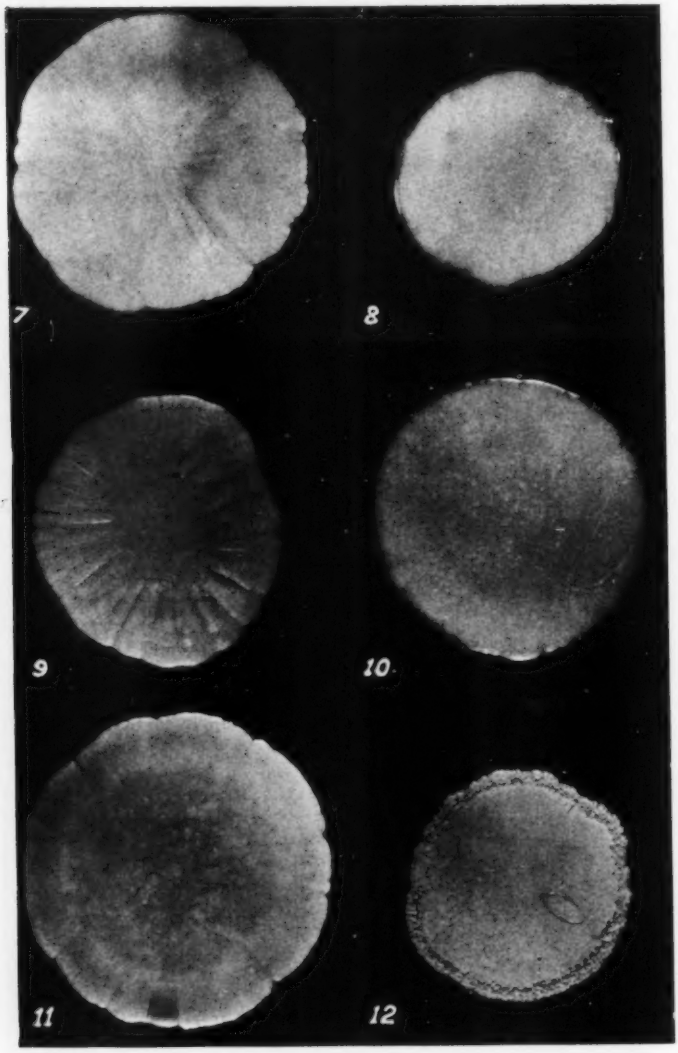
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ABNORMAL MEIOSIS IN *PENNISETUM TYPHOIDES*

I. Desynapsis

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Introduction

The seeds of *Pennisetum typhoides* S. & H. were treated with X-rays and a large number of mutations were reported in the progeny (Krishnaswamy and Ayyangar, 1941). Some of the plants of the progeny were selected for their economic characters. In the progeny of one of these (X–161) in 1947 a few off types were met with. One of these produced proliferations at the basal portion of the panicle. This proliferation appears at the place where a group of spikelets usually is found in normal panicles. In some cases the bristles are clearly seen in a whorl at the base of the proliferation. The proliferated spikelets consist of an outer whorl of three scale leaves enclosing rudiments of leaves and growing point, which in some cases develops into a panicle (Fig. 1). These adventitious panicles appear entirely normal. The rest of the main panicle remained normal without any further proliferations. It was, however, highly sterile. The plant was vegetatively vigorous attaining about 180 cm. height.

The proliferated spikelets resembled an axillary branch or a bulbil in appearance. They remained green and growing even when the main panicle had become dry. They, however, failed to strike root and grow when detached from the head and planted in soil. Next they were given soil when *in situ* by putting soil in half-tile pots and tying up the two as in layering, etc. The soil was kept moist but the proliferations failed to form roots.

The main panicle as already pointed out was highly sterile. Five panicles were analysed for the setting of the seeds and compared with the normal panicles of similar sizes:

Number	Length of panicle	No. of seeds set	
		Sterile panicle	Fertile panicle
1	18 cm.	22	2065
2	18·5 ,,	24	1200
3	17·5 ,,	14	643
4	13·0 ,,	10	674
5	13·5 ,,	5	347

[1] Assistant in Millets Section.

Thus the percentage of seed setting was about 1·5 or nearly 98·5% sterility. The emergence of the anther and stigma was regular as in the normal panicles.

In *E. coracana* Gaertn. as an effect of X-radiation cases in which entire panicles consisted of bulbil-like growths were noted. The mutation was found to be fluctuating in its expression in the same plant with regard to the completeness of the conversion of spikelets into foliar structures. The tertiary and later panicles most often tended to produce more seeds than the more vigorous primary and secondary panicles. Further this mutation was inherited with the normal fertile condition as dominant. In the F_2 and F_3 a large gradation from the entire plant having all the panicles converted to foliar structures to plants having a few spikelets in a panicle converted so, were met with (Krishnaswamy and Ayyangar, 1942). A repetition of this phenomenon was observed in the progeny of one of the irradiated *E. coracana* plant in the later generations. Thus it is quite possible that this proliferation in *P. typhoides* is also the result of X-radiation though it was not met with in the F_1 or F_2 generations. Sometimes such proliferations have been recorded in the untreated populations also as in Sorghum, Rye, etc. Unlike as in *E. coracana*, in *P. typhoides* the affected panicles were highly sterile. The cytological investigations done in temporary acetocarmine smears showed this to be a case of desynapsis. Since this has not been reported in this plant a detailed account of its meiosis is given in the following.

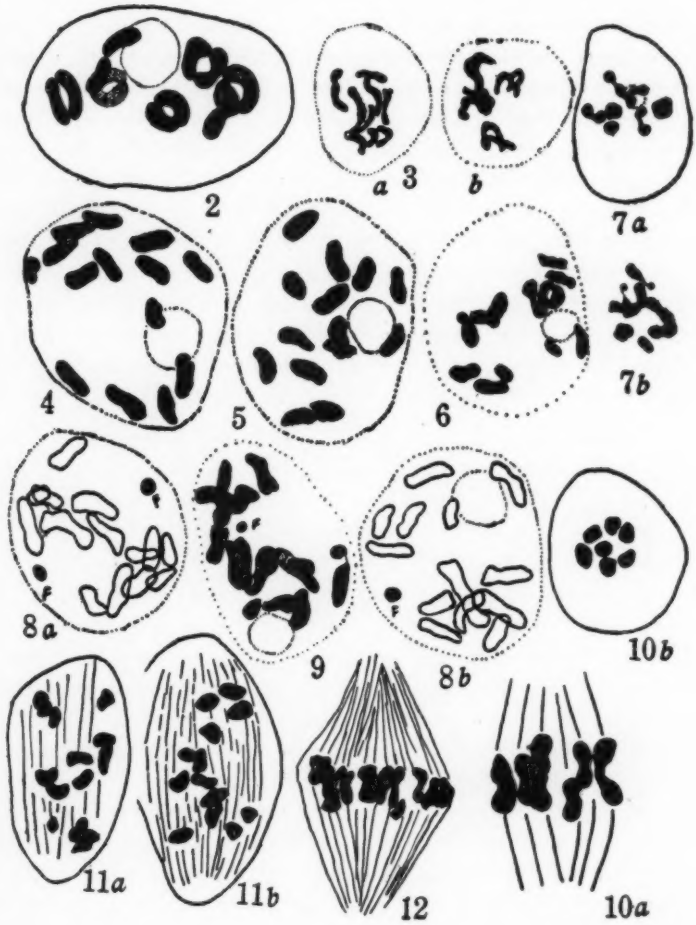
Materials and Methods

The panicles were fixed in Acetic-Alcohol 1 : 3 mixture. Some of the flowers were examined in temporary acetocarmine smears and a few of them were made permanent by McClintock's method. The remainder was cut in paraffin and stained in Gentian-violet-iodine. All drawings were done with a Zeiss microscope with × 15 occular and × 100 oil immersion and Zeiss Zeichenapparat. (Figs. 2 to 36 camera lucida drawings all drawn to a magnification of × 2900, unless otherwise stated. Reduced in reproduction to $\frac{2}{3}$.)

Observations

At zygotene the threads appear double in some cases and rather thick. The synizesis appeared normal with a single nucleolus. At pachytene again the threads appeared paired but whether there were chiasmata formations could not be definitely determined. Conjugation was however found in two pairs very often, while the rest were unpaired. Towards late pachytene already the univalents could be seen and in most of them mitotic divisions were evident. Early diplotene shows the univalents far apart and

completely asynaptic. There was less of contraction. Even here two pairs occasionally appeared as bivalents (Figs. 3 *a* and *b*).



FIGS. 2–12.—Fig. 2. Diakinesis in fertile plant with 7_{II}, the nucleolar pair almost desynapsed Figs. 3–9 meiosis in asynaptic plant 3 *a* & *b*. early diplotene. *a*–showing the split in the univalents into daughter chromosomes. *b*–two bivalents. 4 & 5. Diakinesis with 14_{I}, showing also attachment of the nucleolar chromosomes. 6. diakinesis with 2_{II} 10_{I} (×1600). 7 *a* & *b* with rare multivalent formations at diakinesis. 8 *a* & *b*. Diakinesis with two and one fragments $+14_{I}$. 9. Diakinesis with $_{II}$ and one Sat-chromosome. Fig. 10. A metaphase in normal plant side view, *b*. polar view (× 2100). 11 *a* & *b*. Common Metaphase—1 congression. 12. Rare Metaphase—1 congression.

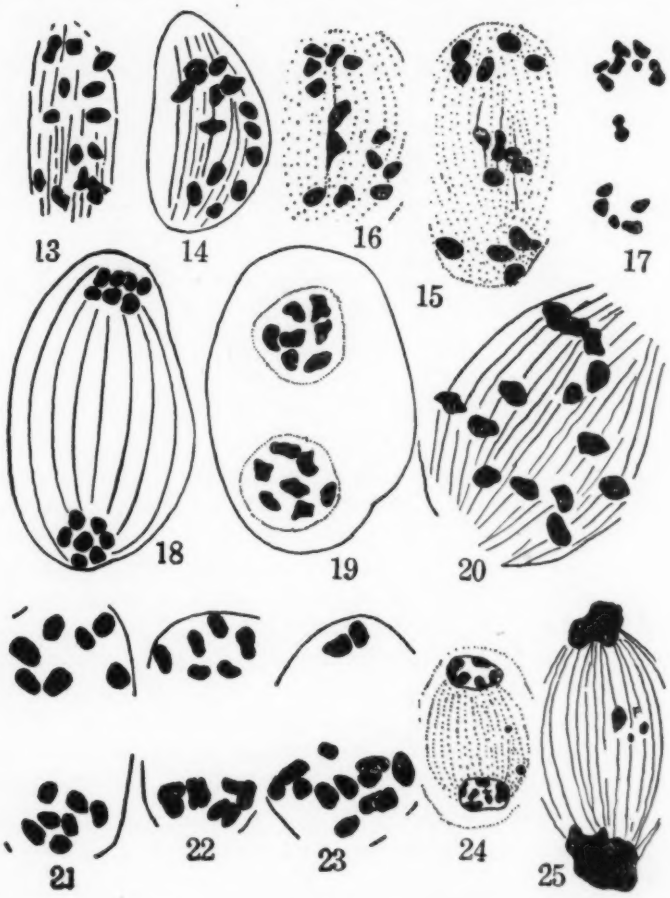
Diakinesis.—At early diakinesis the meiotic chromosomes appeared rather elongate and unpaired. Fourteen chromosomes (univalents) could be counted while in a normal plant at this stage 7 bivalents are regularly formed (Fig. 2). The univalents here were somewhat shorter than the chromosomes in a somatic cell but unlike the meiotic ones (Figs. 4 and 5). There is only one nucleolus formed as a rule and frequently both the nucleolar chromosomes are found attached to the nucleolus but very often only one will be present. Not uncommonly similar chromosomes are seen lying close together. In some of the P.M.C.'s one pair, especially, was often found with extremely terminalised chiasma. Less frequently 2 bivalents are found (Fig. 6). Even in the normal plants a certain amount of desynapsis is met with involving very often the nucleolar chromosomes and less frequently a pair with extremely terminalised chiasmata. A pair of Sat-chromosomes are found unpaired sometimes in the asynaptic plant. Very rarely multivalent formations with 3 and sometimes 4 chromosomes in chains were found (Figs. 7 *a* and *b*). The split into daughter chromosomes which was so evident at pachytene becomes less so at this stage.

Frequency of bivalent formations and desynapsis

	Total No. P.M.C.s	$7_{II}+0_I$	$6_{II}+2_I$	$5_{II}+4_I$	$4_{II}+6_I$	$3_{II}+8_I$	$2_{II}+10_I$	$1_{II}+12_I$	$0_{II}+14_I$
Normal .	173	75	66	32	..	..	..	..	
Asynaptic ..	121	..	..	..	..	..	4	28	89

Metaphase I.—A compact metaphase plate as in the normal plant (Fig. 10 *a* and *b*) is very rarely met with. In such cases a typical bipolar spindle is found (Fig. 12). Usually the univalents are spread out haphazardly on the spindle (Figs. 11 *a* and *b*) which usually is much elongate and bent (Fig. 14). Many times the spindle lacks the convergence at the poles and appears broad and incomplete. A few of the univalents are sometimes migrated to the equatorial region. No definite precedence of division of the bivalent before the univalents moved off could be seen, partly owing to the rare formation of the bivalents at the first metaphase. This may be as Darlington (1937) has pointed out that the unpaired univalents have grouped themselves on the spindle in whatever position they were when the metaphase set on. The univalents do not divide in the first metaphase. They are found to be much contracted almost to the size of the meiotic chromosomes by mid-diakinesis, still they are somewhat elongate more or less sausage shaped. Particular sets of univalents are sometimes found attached by thick traction fibres (Fig. 15).

Anaphase I.—Anaphase of the type found in normal plants (Fig. 18) is rarely met with *i.e.*, a sharp difference between the metaphase and anaphase is wanting. The chromosomes are seen to wander towards the poles quite at random (Figs. 13, 14 and 20). Many of the univalents get included into the poles near which they happened to be at the time of the telophase formation. In some of the cells univalents are found to be at equatorial plate after all the other univalents have moved up to the poles (Fig. 17).



FIGS. 13–25.—Figs. 13 & 14. Typical anaphase, 14 with bent spindle. 15. Univalents with thick traction fibres 16. One bivalent at disjunction. 17. One univalent at equational division? 18. Late anaphase fertile plant showing 7-7 distribution. 19. Interkinesis normal plant (× 2100). 20. asynaptic plant anaphase with probable 7–7 distribution. 21–23 anaphase distribution of univalents 7-7, 6-8 & 2-12. 24. Telophase with 2_y. 25. Telophase with 1_x and 2_y.

Only in very rare cases bivalents were found at the equatorial region undergoing division (Fig. 16).

Owing to the lack of a proper metaphase formation the distribution of the chromosomes to the poles is quite at random (Figs. 21, 22 and 23). Theoretically a distribution from 0–14 to 7–7 is possible. But, however, the tendency is more towards equal distribution than to the formation of a cell with all the fourteen chromosomes. The following gives the frequency of distribution of the chromosomes at Telophase I:

	No. of pollen mother cells with telophasic distribution of chromosomes of								Total P.M.C's
	7/7	6/8	5/9	4/10	3/11	2/12	1/13	0/14	
Normal ..	75	..	..	..	..	..	..	..	75
Asynaptic ..	23	24	13	8	5	2	0	0	75

The daughter nuclei are formed normally with nuclear membrane. Cytokinesis is complete. In some cases nucleoli are also found.

It is quite common for one or two chromosomes lagging at first anaphase, and frequently, they are not included in the daughter nuclei. They are found left on the spindle at various positions according to their movement. Very rarely restitution nuclei are found at Telophase I (Figs. 26, 27),

Second division.—Metaphase II is more regularly formed than Metaphase I (Fig. 30). Enough number of P.M.C.'s of the anaphase of second division were not available to make a detailed study. The distributions in the few cases counted, show that the univalents undergo equational divisions and the segregations are complete. Rare instances of chromatin bridges with probably fragments were noticed (Fig. 31). The tetrad formation is regular (Fig. 34). Supernumerary divisions of the cells or the formation of restitution nuclei were not met with. Well formed, walled-in pollen grains resembling the normal ones are formed. They are highly sterile. Pollen grains stained with iodine and counted for fertility gave 230 empty and only 6 full ones indicating nearly 98% sterility. The pollen grains are smaller than in the normal plates:—

Diameter of pollen grains

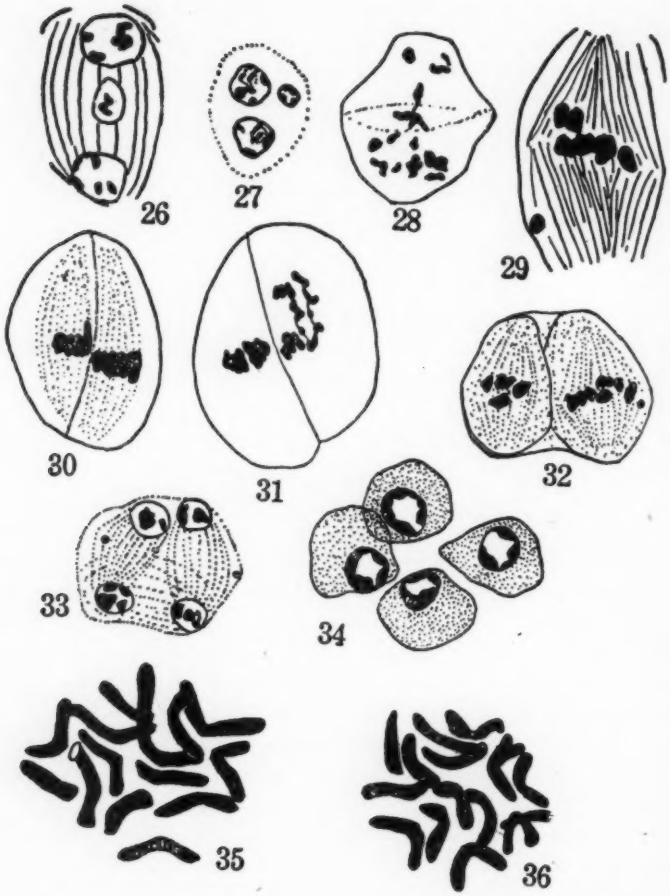
No. of divisions of the Oc. micrometer	9	10	11	12	13	14	15	16	17	18	19	20	Total
Normal ..	..	..	..	..	..	..	..	..	7	19	2	22	50
Asynaptic ..	3	9	11	2	10	3	8	4	..	..	..	..	50

Fragments.—At diakinesis (Figs. 8 *a* and *b*) and at the first metaphase, anaphase and telophase one to two extra chromatin bodies were found in the spindle (Figs. 24, 25 and 29). These were always smaller than the univalents, spherical and most often lay at a different plane than the other chromosomes. They are found on the spindle after the univalents had migrated to the two poles. These were frequently left out of the daughter nuclei at first division. In some cases they were found still on the spindle after the formation of the daughter nuclei in the second division (Figs. 32 and 33).

Discussion

Asynapsis (or asyndesis or desynapsis) has been noted in a number of plants, sometimes in natural progenies and sometimes as an effect of treatment chemical or mechanical, resulting in lack of pairing. Darlington (*l.c.*) summarises the " causal relationships of different kinds of non-pairing " as (1) Genotypic abnormality (asynaptic mutants), (2) Lack of genotypic adaptation to structural conditions (new fragments), (3) Structural differences (structural hybrids) and (4) Numerical abnormality (Trisomics, Triploids, etc.). The first of these affects all chromosomes, there is lack of precocity, reduced pachytene pairing to a variable extent resulting in insufficient numbers of chiasmata for regular metaphase pairing. Practically all the reported cases more or less conform to this description. Levan (1940) would however, make the term ' asynapsis ' more comprehensive. He states, " The term asynapsis usually denotes a whole group of phenomena characterised by decreased chromosome pairing during the first meiotic division. Asynapsis can be modificative or genotypic. Temperature, age, or chemical action may cause asynapsis. Evidently failure of chiasma formation is the typical response if a certain stage of meiosis is subjected to external irritation. Genotypic asynapsis may be due to lacking of chromosomal homology, that is the case in numerical and structural hybrids and haploids, and it may be true gene action." Li, *et al.* (1945) suggest that the word " asynapsis " be used to " only for those cases in which synapsis is absent during the first meiotic mitosis as originally defined by Randolph (1928) " and the term " desynapsis " be used for all such cases of genetically controlled failure of metaphase pairing in which synapsis does take place between pachytene and diplotene.

Definite monogenic control of the asynapsis has been found in *Zea* (Beadle and McClintock, 1928; Beadle, 1930), in wheat (Li, *et al.*, 1945), *A. amplectens* (Levan, *l.c.*), in *Pisum* (Koller, 1938), in *N. tabacum* (Clausen, 1931) and *N. sylvestris* (Goodspeed and Avery, 1939), in *Oenothera* (Catcheside, 1939). Li, *et al.*, find in wheat stable and unstable types of



FIGS. 26–37.—Fig. 26. Interkinesis with restitution nucleus (6–3–5). Fig. 27. Resititutional nucleus formed to one side of the spindle, × 1600. Fig. 28. Delayed disjunction? × 1600. Fig. 29. Fragments at M–I. Fig. 30. Second metaphase. Fig. 31. Second anaphase with chromation bridges. Fig 32. Metaphase II, with one fragment. Fig. 33. Telophase II with 2_n. Fig. 34. Tetrad (32–34 × 1600). Fig. 35. Metaphase plate from root with 14 chromosomes normal plant. Fig. 36. Somatic plate from $2n + 1$ plant. (35 & 36 × 4,900).

desynapsis. They find that probably in wheat there are " modifying genes that control the degree of manifestation of synapsis on the one hand and constancy of the bivalent frequency is controlled by another set of modifying genes ". Further the desynaptic gene is also affected by temperature

in its manifestation being most effective when temperature is lowest below 10° C. and with rise of temperature complete pairing may be found when an optimum is reached. Huskins and Hearne (1935) adduce the asynapsis to loss of specific chromosomes in dwarf wheat and oats. They find that lack of pairing is correlated with premature splitting of chromosome threads and irregular contraction. Levan (*l.c.*) has found very strong and intimate pairing of chromosomes at pachytene but at diplotene he found that no real chiasma had been formed and owing to the increasing contraction the relational spirals uncoil and the chromosomes fall apart. Even at diplotene the split between daughter chromosomes was evident thus proving Darlington's (*l.c.*) statement that premature divisions lead to reduced pachytene pairing and do not produce chiasmata. This reduced precocity also produces a reduced contraction of the chromosomes at metaphase giving rise to the long " semi-mitotic " chromosomes. Such chromosomes have been noted in *A. amplectens* (Levan, *l.c.*), in *Wikstræmia* (Fagerlind, 1940), in *Pisum* (Koller *l.c.*). Similar semi-mitotic chromosomes are found in the present case also, these however, undergo in many P.M.C.'s a certain amount of contraction towards metaphase, while in others they are found elongated. According to Fagerlind (*l.c.*) the failure to contract is probably a cause for the asyndesis. " Die Asyndese bei vielen Agamogonen ist sehr wharscheinlich durch die ' Somatisierung ' der Meiosis bedingt. Ist diese stark, so werden Teilungen erhalten, bei denen die Chromosomen somatische Gestalt haben; ist sie schwach, so behalten die Chromosomen ihre meiotische Gestalt, aber es trit asyndese ein. Ist die ' Somatisierung ' sehr schwach so wird sie etwas stärker, sowird die Asyndese total."

The metaphase is similar to the other cases reported. In *A. amplectens* however, there is a normal development of the spindle though the asynapsis is complete (Levan, *l.c.*). In the present instance there are very few cases of bivalents at metaphase plate seen, evidently due to further dissolution of the chiasma in the two pairs that tend to form bivalents, between diakinesis and metaphase. The spindle is varying, a well formed one occurring only when the chromosomes are congregated at the equatorial plate. Supernumerary and even split spindles have been noted in *Oryza* (Ramanujam and Parthasarathy, 1935). Levan finds that this feature of forming regular spindles in *A. amplectens* is owing to the command the spindle gains over the univalents and due to the centromere in both univalents and bivalents being same structurally. They must all be polarized and capable of auto- as well as co-orientation.

The anaphase and telophase are regularly gone through. In *A. amplectens* on the other hand the Anaphase I is omitted so that with the Anaphase II

dyad pollen with somatic numbers are formed. In *Oenothera* the univalents split invariably at Anaphase I (Catcheside, *l.c.*). Anaphase I is irregular in many of the reported cases but in *Oenothera* the Anaphase II is irregular resulting in unequal distribution of the chromosomes. The tetrad formation in *P. typhoides* is regular and unlike in other cases no restitution nuclei are formed. Fragments are found but these seem to be lost thus leading to certain amount of chromatin loss. The origin of the fragments and certain multivalent associations occasionally met with could not be clearly traced. In *Wikstræmia* during Metaphase I small chromosomes (Klein-chromosomen) have been met with and in *Zea* ' diminutive ' chromosomes assumed to have arisen by fragmentation have been noted. Koller in *Pisum* has recorded structural hybridity—an inversion in one chromosome.

This sterility in the microspores is attended with female sterility also. It appears as though on the female side also it is rather high since the seed setting is very poor. These plants were surrounded in the field by numerous normal plants and on two panicles deliberate dusting of pollen was done, still the seed setting was poor. Beadle and McClintock (*l.c.*) have recorded a similar experience in *Zea*.

The progeny of the asynaptic plants have been tested and found to give plants of varying numbers of chromosomes such as polysomics, triploids, etc., [*Zea*, wheat, *capsicum* (Pal and Ramanujam, 1940), *Pisum*, etc.]. Roy and Powers (*l.c.*) have stressed the importance of the study of such asynaptic forms in the breeding programme.

The seeds obtained from this plant in *Pennisetum* have been sown and one of the seedlings tested gave a $2n + 1$ number (Figs. 35 and 36). Unfortunately this particular plant was weak and died. Other plants are being raised for study. Whether this sterility is inherited or not is to be determined only by the F_2 progeny.

Conclusion

The terms asynapsis, asyndesis and desynapsis seem to have been used by the different authors as synonyms. In many of these plants there are recorded evidences of pairing having taken place. According to Li and Pao they clearly come under the term desynapsis as defined by them. In the *Pennisetum typhoides* plant under consideration there are definite evidences of conjugation having taken place at least in one or two pairs. The bivalents desynap early so that by diakinesis there is complete failure of pairing. The rare occurrence of bivalents seems to support this view. Thus this also forms a case of desynapsis.

Summary

(1) In the progeny of X-rayed *P. typhoides* S. & H. one plant having some basal spikelets proliferated into foliar structures was found to be also male and female-sterile. This sterility was traced to desynapsis.

(2) The meiosis of this desynaptic plant is fully studied. The prophase indicates pairing to have taken place at least in two pairs. At diakinesis the desynapsis is found to be nearly complete. In about 26% of cases one or two bivalents are formed. Certain amount of desynapsis is found in the fertile plant also. By Metaphase I even this percentage gets reduced so that at early Anaphase I disjunction of bivalents are rarely seen. The univalents rarely form a metaphase congression. The spindle is often bent. Restitution nuclei were not found.

(3) In the distribution of the univalents to the poles at Telophase I a greater tendency towards equal distribution is found.

(4) Fragments at diakinesis, Metaphase and Anaphase I and at Anaphase and Telophase II were found. Rare cases of multivalent formations were met with.

(5) In the progeny one plant with $2n + 1$ number was met with.

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Fig. 1. Panicle of asynaptic plant showing proliferation at the basal portion and one of them with a panicle. ½ Nat Size.

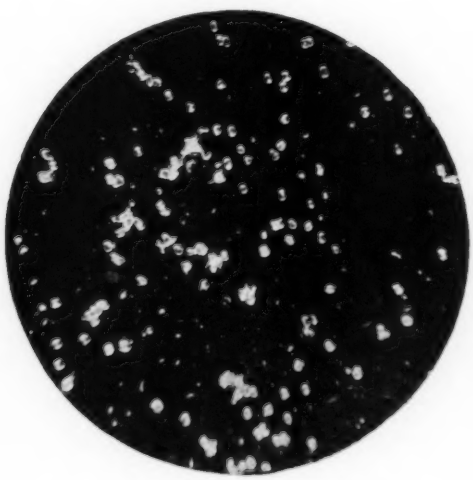

Fig. 37. Pollen grains desynaptic plant × 501.

EARLY STAGES IN THE DEVELOPMENT OF *SACCULINA* SP., PARASITIC ON *NEPTUNUS SANGUINOLENTUS* FROM MADRAS

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Introduction

In the course of the study of the anatomy of *Neptunus sanguinolentus*, the author found on 27th of October 1947, a female crab, measuring 5 cm. across the carapace, parasitised by two forms belonging to the genus *Sacculina*. These were attached, one close behind the other, to the ventral side of the third and fourth abdominal segments. Though the crab as well as one of the parasites was dead the other parasite was alive and was studied *in situ* in the laboratory. As this parasite happened to be a gravid female it was possible to rear nauplii and cypris stages of development. But no suitable living host being available, artificial infection could not be attempted in order to follow the life-cycle further. Detailed descriptions of the nauplii and cypris of this important Rhizocephalan parasite are few, and the best not so full as could be desired. As the author felt that a full description of the nauplius and cypris may be useful in completing the life-history when these forms are encountered in the plankton, these stages of Sacculina, infecting the common edible crab *Neptunus sanguinolentus* are described here.

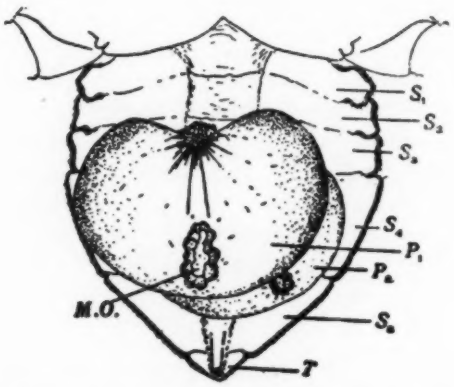


Fig. 1. Ventral view of the abdomen of *Neptunus sanguinolentus* with the two parasites attached to it, × 4.

Material and Methods

Of the two parasites found on the same crab, the larger, which was alive, was attached to the third segment of the abdomen. It measured 13 mm. long from the mantle opening to the hind end and 19 mm. across. (The smaller dead parasite was 9 mm. long and 14 mm. broad.) The live specimen with its dead host was transferred to a bowl of clean sea-water at 11 A.M. and observed under a binocular. The mantle containing the body was seen to pulsate regularly. The pulsations were timed with a stop watch and it was found that in the first two hours the pulsations took place 9, 9, 8, 7, 7, 7, 7 and 6 times per minute during the eight periods of 15 minutes. During the next ½ hour, only 6 contractions per minute took place and only 5 contractions followed in the succeeding 30 minutes. Thereafter the mantle pulsated only 4 times per minute for the next two hours and stopped afterwards at 4 P.M. During each contraction the bag-like body decreased in length as well as breadth and recoiled to its former size. Of the 83 contractions observed, most involved both sides of the body of the parasite. But during some of the contractions, one side moved alternately.

During each contraction of the mantle, a large number of minute nauplii were ejected out of the mantle cavity through the mantle opening and each emission appeared like a tiny white cloud. It is possible that the pulsations which were frequent at first became slower as the stock of the nauplii ran down, which could be inferred from the fact that only very few nauplii emerged out of the mantle cavity towards the end and the body of the parasite became more transparent. As it was difficult to keep the parasite alive when the host was dead, the parasite was fixed. The nauplii were transferred to clean sea-water and studied.

Next day at 10-30 A.M. most of the nauplii were alive but one or two cypris stages were detected. The nauplii which were studied showed three different stages of development, probably reached after each moult, though no nauplius was actually observed in the process of moulting, by the author. Some of the older nauplii showed indications of a fourth segment. The nauplii were under close observation for about 4 hours. As after a brief interval a few cypris forms were found it is probable that the transformation from nauplius to cypris was sudden and brief. The next day the total number of cypris larvæ produced was 15. Several of the nauplii and cypris larvæ were fixed, mounted and studied.

Nauplius Larva

The nauplius larva is 0·2 to 0·23 mm. long when measured from the front margin of the carapace to its posterior tip, and 0·12 to 0·15 mm. in

breadth measured between the frontal horns. The carapace is triangular in shape with the two sides curved outwards. The frontal margin is produced laterally into two horns, each of which ends in two points. Between these points open the ducts of the cement glands which are easily made out when stained with borax carmine. At the posterior end of the carapace

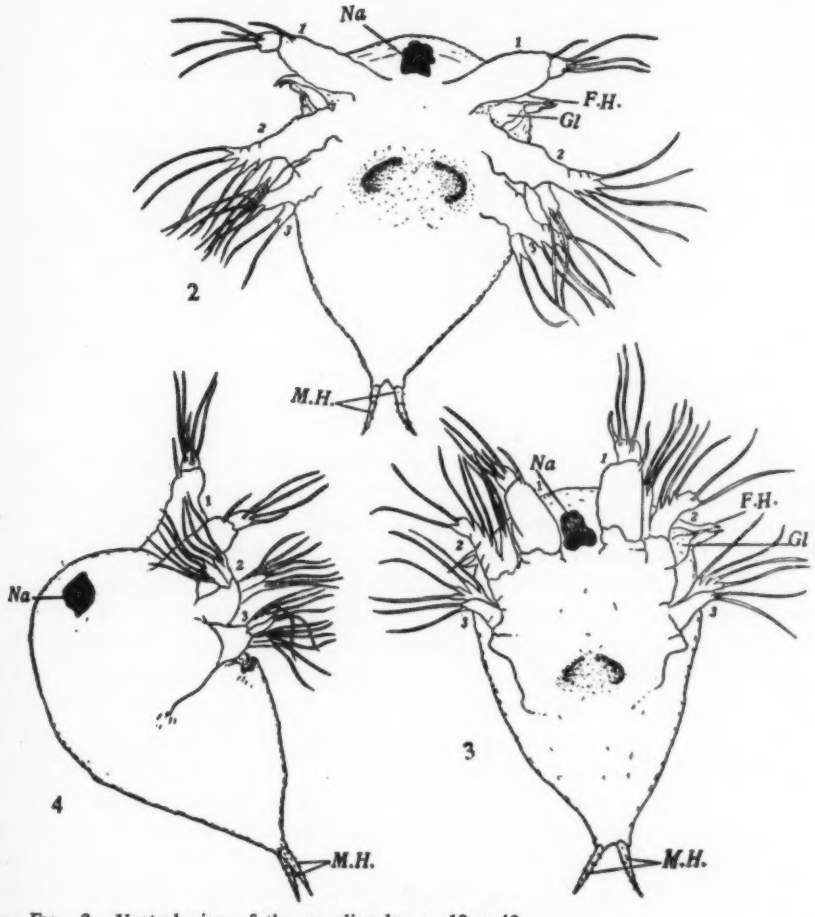


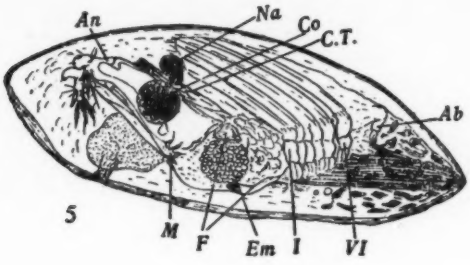
FIG. 2. Ventral view of the nauplius larva, 10 × 40.
FIG. 3. Ventral view of the nauplius larva after one moult, 10 × 40.
FIG. 4. Lateral view of the nauplius larva, 10 × 40.

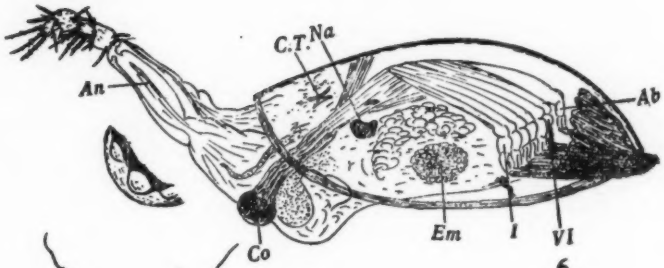
there are two median horns which bear minute spines. The nauplius eye is situated in the middle of the anterior portion of the larva about 5 to 10 μ

behind the anterior margin of the carapace. Three pairs of appendages of which the two posterior are biramous, are present. The first pair of appendage or *first antenna* is four segmented, of which the two proximal are short and rectangular. The third segment is very stout and is thrice as long as the other segments and twice as broad as the distal segment, which is the smallest among the four. On this distal segment there are three long setæ and one short spinous seta. A short spinous seta is present on the distal end of the third segment. Unlike those of the larvæ of the other Cirripedes the protopodites of the *2nd and 3rd pair of appendages* are devoid of masticatory hooks. The protopodites are two segmented. The exopodites of the 2nd pair possess five articulations, each with a single seta. There are three setæ on the endopodite which is only half the size of the exopodite. The exopodites of the third pair possess four articulations and four setæ, and there are two setæ on the endopodite. All the setæ on the appendages appeared like spines, unlike those of some of the other Cirripedes whose nauplii have appendages with plumose setæ. A few nauplii collected appeared slightly older. They possessed a distinct segment in addition to the three belonging to the nauplii. But they possessed only bud-like rudiments of appendages.

Cypris Larva

Like the cypris larvæ of other Cirripedes, this also has a bivalved shell. The Cypris larva is 0·5 mm. in length from the anterior end to the posterior end and 0·22 to 0·25 mm. in breadth. The *first antenna* is uniramous, very stout and four jointed. The first segment of the antenna starts from a point in front of the eye. When at rest, this segment is directed backwards. It extends as far behind the eye as the portion in front of it. The second segment is very stout and rectangular, with the distal end narrower. This segment is kept folded on the first segment, on the ventral side, so that it is directed anteriorly. The anterior end of the third segment of the antenna is flat and disc-shaped and is used for fixation. In the centre of the disc, a star-shaped grove may be clearly seen and at the margin there is a fringe of short spine-like setæ. The terminal segment is hooklike and is attached on the ventral side of the distal part of the previous segment. It ends in a sharp stout spine and bears four plumose setæ of varying lengths. But all of them are longer than the segment itself. *The second antenna* is absent. The *mouth* of the cypris is situated on the ventral side just behind the base of the second segment of the antenna. It is overhung by a short labral plate. The mouth parts are not only rudimentary but small. The *mandible* is made up of two palp-like projections, of which the anterior is stouter than the posterior. Some very minute setæ can be noted at the tip of the former.





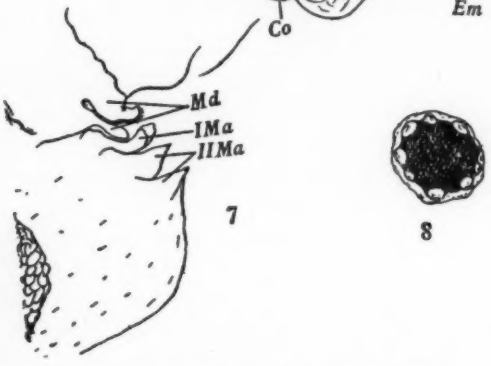


FIG. 5. Lateral view of the Cypris larva, 5 × 40.
FIG. 6. Lateral view of the Cypris with the antennæ stretched out, 5 × 40.
FIG. 7. Mouth parts of the Cypris, 30 × 40.
FIG. 8. Compound eye of the Cypris, 10 × 40.

The *first and second pair of maxillæ* are less conspicuous, being mere projections. The tip of the first maxilla is round, while that of the second is acutely pointed.

THORACIC APPENDAGES

There are six pairs of these biramous limbs. Each is attached by a bundle of muscles which is sharply turned forwards. The protopodite is

of two cylindrical, unequal segments, the distal being longer. The exopod and endopod are similar and consist of two joints of which the proximal bears at its tip a stout spine-like bristle. The distal joint is leaf-like and bears at its posterior margin four long setæ, which are longer than the rest of the leg. The posterior tip of the body ends in a slender, short *abdomen*, which ends in a caudal fork. The proximal segment of the abdomen is cylindrical. Each division of the caudal fork bears two long setæ at its tip.

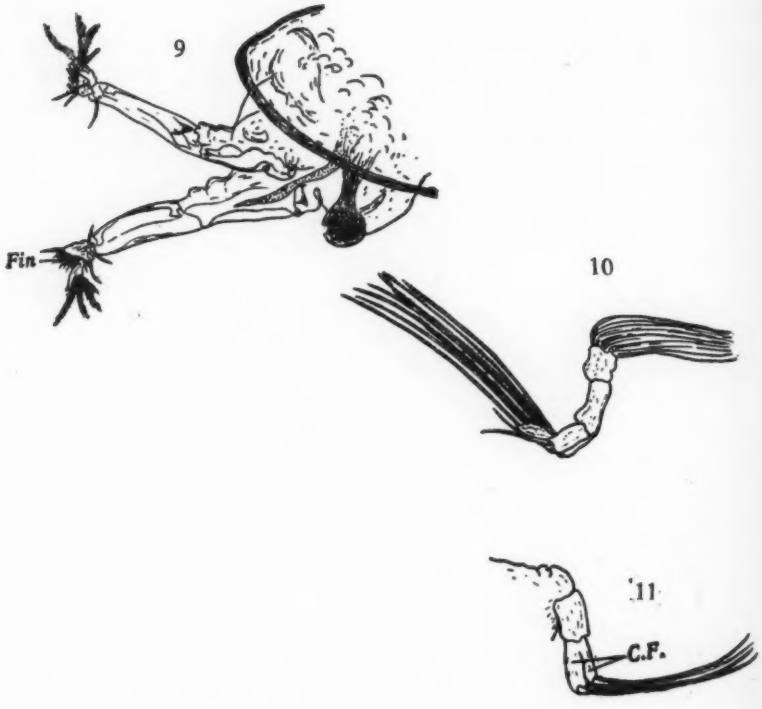


Fig. 9. First Antennæ of the Cypris stretched out, 5 × 10.
Fig. 10. Third thoracic appendage of the Cypris, 10 × 40.
Fig. 11. Abdomen of the Cypris larva.

At about one-third the length from the anterior end of the body of the cypris is situated the paired, round compound eye. It is 50 μ in diameter. Eight lens units were made out along the margin in some, while, in some others only seven were counted. Just towards the dorsal side of this eye and almost touching it persists the median nauplius eye in an elongated condition.

The cypris larvæ were found to be swimming about actively. When swimming the muscles of the appendages contract in such a way that the appendage is drawn forwards rotating the tuft of long setæ to a position perpendicular to the long axis of the body. While the muscles relax, they do so suddenly. The appendage is thus kicked backwards and the setæ sweep backwards and upwards displacing the water effectively backwards and propelling the larva forwards.

General Remarks

The same crab being parasitised by two parasites of the same species has not been recorded by various authors. Further their attachment to the third and fourth abdominal segments is unusual as G. Smith has described the junction of the thorax and abdomen as the place of attachment. According to Y. Delage and G. Smith the nauplius of Sacculina undergoes four moults in five days, before reaching the cypris stage. In this specimen, however, the cypris stage was seen about 24 hours after the emission of the nauplii from the brood pouch of the parent. The quicker development is probably due to the tropical conditions. The occurrence of both the median as well as the paired compound eyes in the present form is noteworthy, since Delage and Gissler have both found only the median eye in the cypris of the parasitic Cirripedes, though compound eyes have been found in Cypris larvæ of free living Cirripedes.

The descriptions Geoffrey Smith has given of twelve genera of Rhizocephala collected from Gulf of Neapel include a list of host crabs on which *Sacculina rotundata* has been found. Judging from the descriptions and figures given by Smith and Delage, the present form differs from *Sacculina rotundata* in the presence of both the median eye and the compound eye in the cypris larva, the attachment of the parasites on the third and fourth abdominal segments of the host and the more rapid development of the nauplius into the cypris. In view of these differences it may not be justifiable to identify the present form as *Sacculina rotundata*. Yet because this particular species has been recorded from the genus Neptunus, the present form may be provisionally identified as *Sacculina rotundata* pending fuller study of its complete life-history.

Acknowledgements

I wish to take this opportunity to thank Dr. C. P. Gnanamuthu, M.A., D.Sc., F.Z.S., Director, Zoology Laboratory, University of Madras, for his invaluable guidance and criticism throughout the course of this work. The author also wishes to acknowledge his perusal of Mr. A. I. George's

unpublished thesis on " The Development of the Rhizocephalan Parasite *Heterosaccus ruginosus* (Boschma) and Effects of Parasitic Castration on *Neptunus pelagicus*."

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Key to Lettering

Ab.	.. Abdomen	*Md.*	.. Mandible
An.	.. Antenna	*M. H.*	.. Median horns
Co.	.. Compound eye	*M. O.*	.. Mantle opening
C. F.	.. Caudal furca	*Na.*	.. Nauplius eye
C. T.	.. Chitinous tendon	P_1.	.. Bigger sacculina attached to the 3rd abdominal segment
Em.	.. Embryonic cells of ovary		
F.	.. Fat globules	P_2.	.. Small sacculina attached to the 4th abdominal segment
F. H.	.. Frontal horn		
Fin.	.. Disc for fixation	S_1–S_5.	.. Abdominal segments 1 to 5
Gl.	.. Glands of the frontal horn	*T.*	.. Telson
M.	.. Mouth parts	1–3	.. Nauplius appendages
I Ma.	.. First Maxillae	I–VI	.. Thoracic appendages
II Ma.	.. Second Maxillae		

THE INTERRELATION BETWEEN THE VARIOUS METABOLIC MECHANISMS IN UNSTRIATED MUSCLE

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Received October 4, 1948

Singh and Singh (1948 *a*, *b*; 1949); Singh (1949), have described several metabolic mechanisms in unstriated muscle. In the present research, an attempt has been made to find the interaction and interrelation between these mechanisms.

Experimental procedure was as described previously. The muscle was stimulated by alternating current in these experiments.

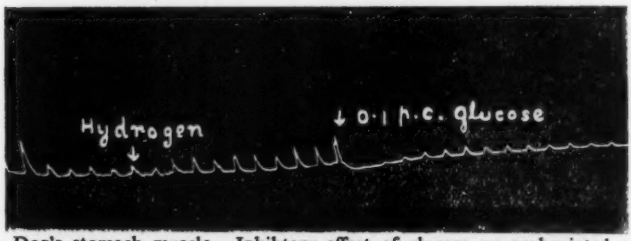

Fig. 1. Dog's stomach muscle. Inhibtory effect of glucose on asphyxiated muscle.

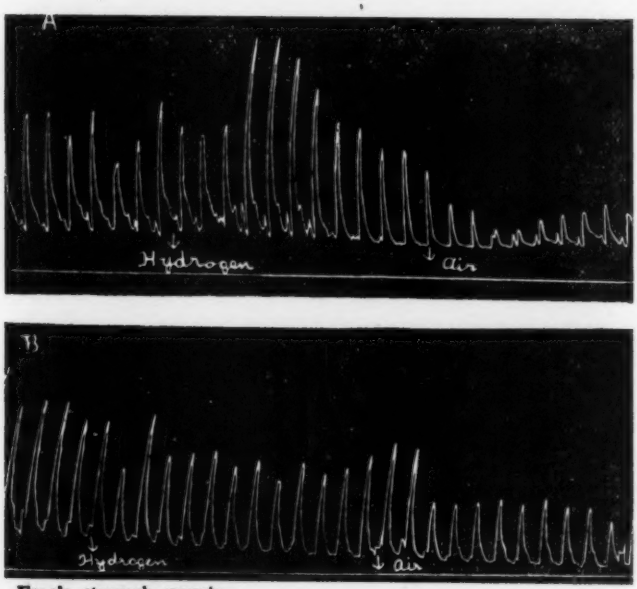

Fig. 2. Frog's stomach muscle.

A. Effect of asphyxiation in the absence of glucose.

B. Effect of asphyxiation of the above muscle in presence of glucose, 0·1 per cent.

Results

Asphyxial increase in excitability.—Glucose 0·1–0·2 per cent. partially inhibits the asphyxial increase in excitability (Figs. 1, 2). This inhibitory action is temporary and the muscle recovers, glucose now having an opposite action. Correspondingly, the asphyxial increase in excitability is increased by agencies, that depress the formation of lactic acid, such as iodoacetic acid (Fig. 3) and a rise in acidity upto pH 6·5. This may

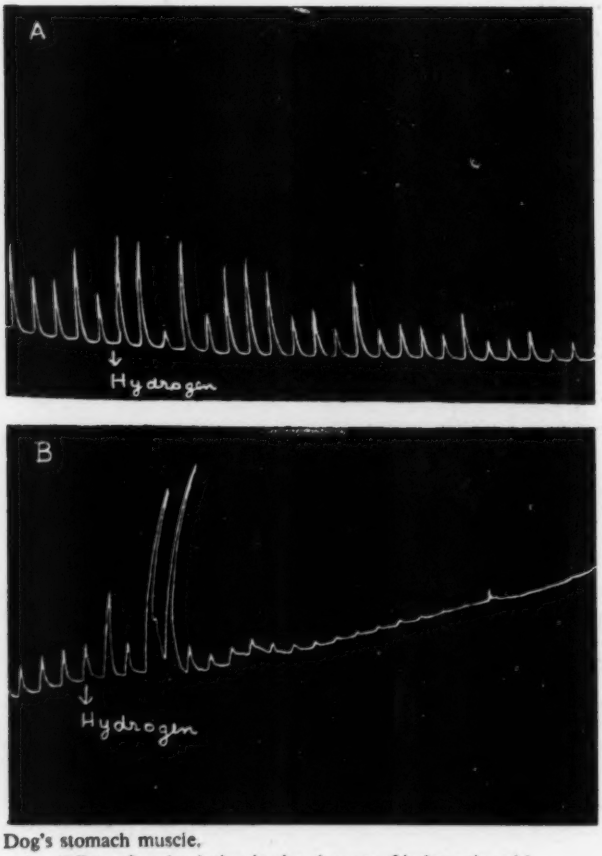

Fig. 3. Dog's stomach muscle.
A. Effect of asphyxiation in the absence of iodoacetic acid.
B. Effect of asphyxiation in the presence of iodoacetic acid; dog's muscle passes into contraction under iodoacetic acid.

enable the muscle to withstand asphyxia better in acid solutions (Fig. 4*a*) or if poisoned with iodoacetic (Fig. 4*b*). These results are similar to those

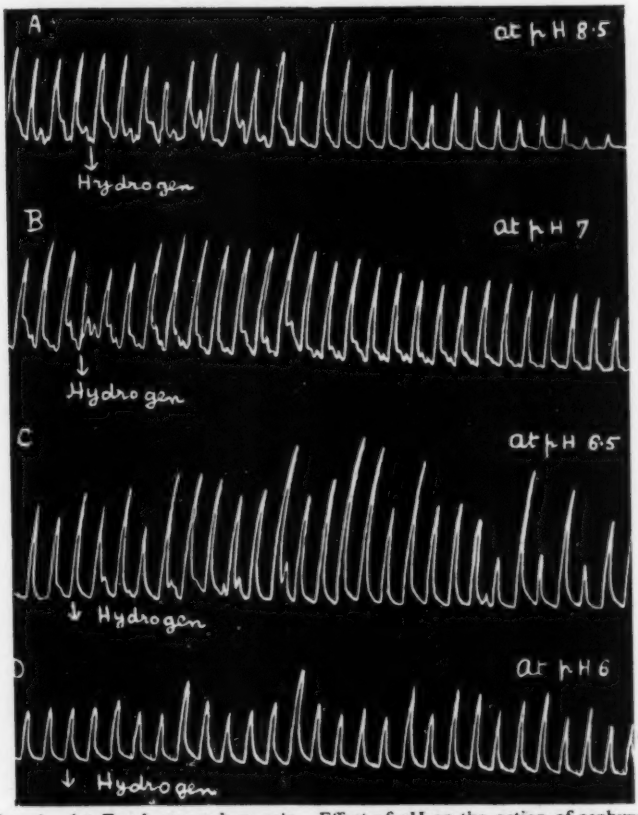

FIG. 4. (*a*) Frog's stomach muscle. Effect of pH on the action of asphyxia.

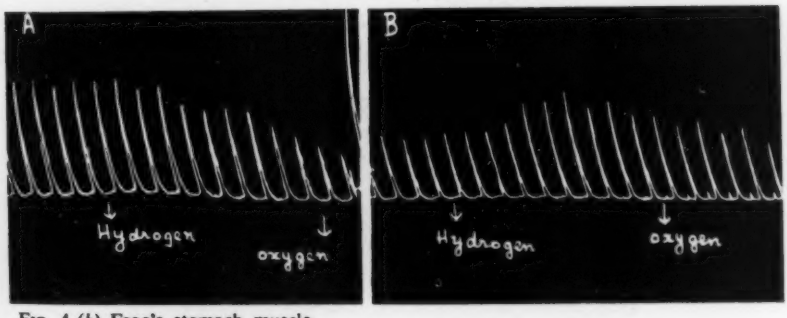

FIG. 4 (*b*) Frog's stomach muscle.
A. Effect of asphyxiation in the absence of iodoacetic acid.
B. Effect of asphyxiation in the presence of iodoacetic acid.

found in the central nervous system. Therein the effects of anoxia and hypoglycæmia are closely similar and supplementary to one another. Hypoglycæmic convulsions are induced more readily in the presence of anoxia and the effects of latter are more severe if the blood sugar is depressed (Best and Taylor, 1945).

Anaerobic glycolysis also produces asphyxial increase in excitability. This is shown by the action of iodoacetic acid which suppresses it and glucose, which increases it (Fig. 5). Also on addition of glucose to an

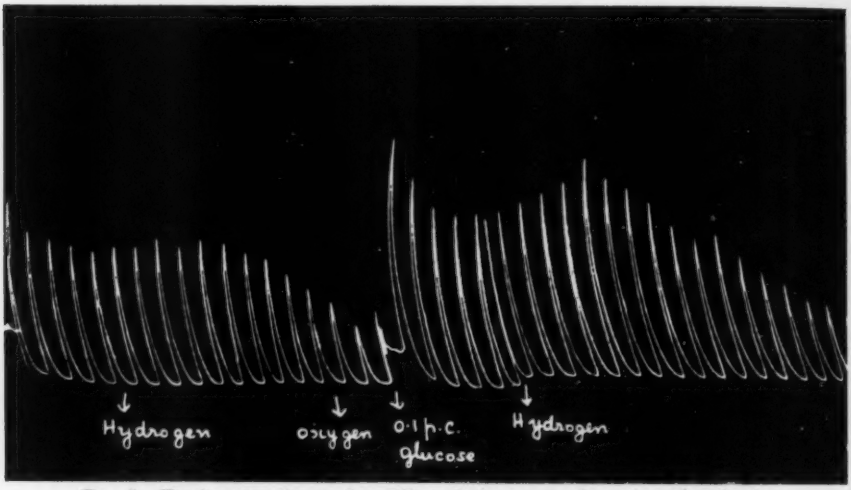

FIG. 5. Frog's stomach muscle. Effect of glucose on the action of asphyxia.

asphyxiated muscle, the response may increase above normal before declining to a steady state (Figs. 6, 7). This is in agreement with findings in other parts of the body. In the carotid body the asphyxial stimulation is prevented by iodoacetic acid (Best and Taylor, 1945). This, therefore, is

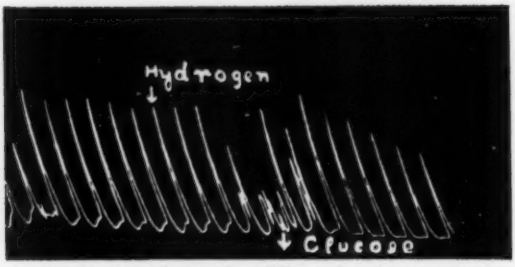

FIG. 6. Frog's stomach muscle. Effect of asphyxia and glucose.

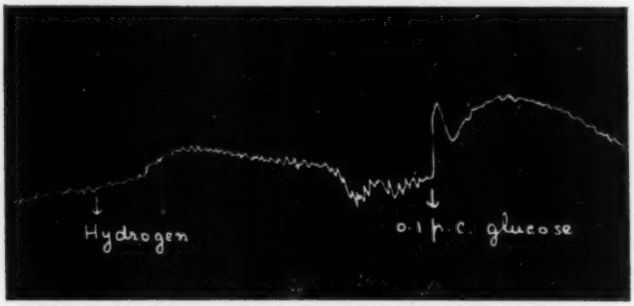

FIG. 7. Fowl's gut. Effect of asphyxia and glucose on tone.

different from the asphyxial stimulation in the central nervous system, where glucose has an inhibitory effect.

Thus in the muscle there are two antagonistic anaerobic mechanisms. Their relationship is shown by the following observations: If the muscle is completely exhausted, then glucose has always a stimulatory effect. If the asphyxial increase in excitability begins to decline, then also glucose has a stimulatory effect. This suggests that the glycolytic mechanism succeeds the first one and if the latter is feebly acting or not at all, then the glycolytic mechanism is active from the start, as shown by the action of glucose.

The disappearance of the inhibitory effect of glucose with time and its absence if the muscle is soaked for some time, shows the utilisation of glucose if present in the saline. These effects of glucose resemble those on the heart muscle; glycogen appears to be used only as an emergency fuel, that is when heavy work is undertaken. Under certain conditions only a small fraction of the oxygen consumed by the heart can be accounted for by the oxidation of carbohydrate, but with a high blood sugar the respiratory quotient may be 0·95 or 1·0 indicating a high carbohydrate utilisation. In the brain also, there is a direct relationship between the level of the blood sugar and quantity of glucose used (Best and Taylor, 1945).

The reverse process of taking over by the non-glycolytic from the glycolytic mechanism may be studied by the action of iodoacetic acid or acid solutions (pH 6). This response may increase immediately or after a preliminary depressant effect lasting from 10 to 60 minutes (Fig. 8). Their effects may be permanently depressant, showing exhaustion of the non-glycolytic mechanism. The preliminary depressant effect of iodoacetic acid or acid solutions shows that the non-glycolytic mechanism starts to function after the glycolytic mechanism becomes less active.

The anaerobic utilisation of glucose appears to happen in two ways. When glucose is added as the muscle is being asphyxiated there may be

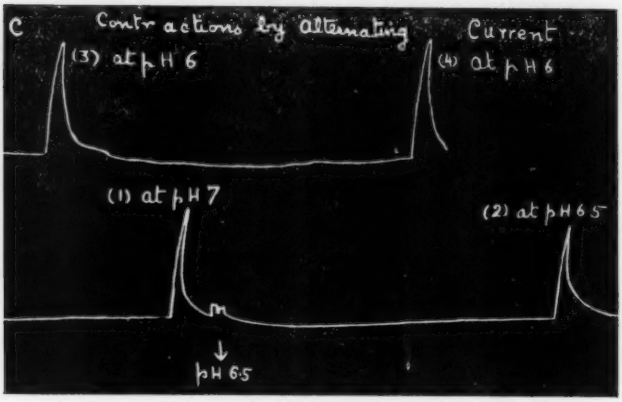

FIG. 8. Frog's stomach muscle. Effect of pH 6.

an immediate increase in the response followed by a decline (Figs. 6, 7); or the response increases gradually to that state (Fig. 9). The second state

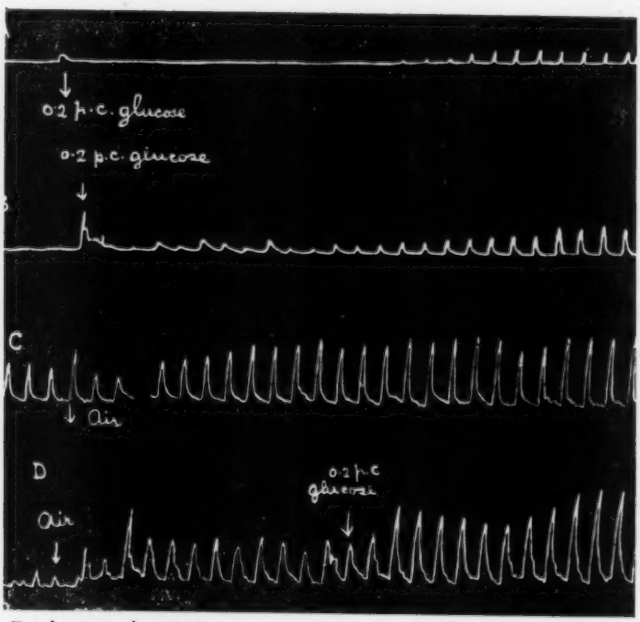

FIG. 9. Frog's stomach muscle.
A. Effect of glucose on muscle treated with cyanide (1 in 10,000).
B. Effect of glucose on muscle paralysed by asphyxia.
C. Effect of oxygen on the muscle in B.
D. Effect of glucose in the presence of oxygen. Muscle asphyxiated prior to oxygen.

can be produced with certainty by first completely exhausting the asphyxiated or cyanide treated muscle and then restoring the response by glucose. In the first instance glucose must have been utilised in conjunction with some substance, which becomes exhausted, the combination being more potent than glucose alone. In the steady state the response is always less than that in the presence of oxygen; but in the other state it may be greater than normal indicating that the glycolytic asphyxial increase in excitability is produced by the above combination.

There are thus two glycolytic anaerobic mechanisms. When the muscle is in the steady state described above, oxygen usually improves the response. Similarly in the presence of oxygen, glucose usually improves the response. Thus the anaerobic glycolytic mechanism during the above state is similar to the aerobic one.

Introduction of oxygen may produce a temporary inhibitory effect on asphyxiated muscle which has been treated with glucose (Fig. 10). This

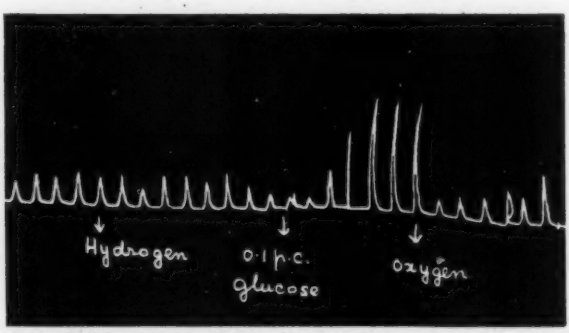

FIG. 10. Dog's stomach muscle. Inhibitory action of oxygen on glucose treated asphyxiated muscle.

glycolytic anaerobic mechanism is, therefore, the same as that glycolytic one, which produces asphyxial increase in excitability. In this case, therefore, the anaerobic utilisation of glucose is different from its ærobic usage. These results are similar to those found by workers on striated muscle. Some observers maintain that the aerobic utilisation of glucose is similar to its anaerobic utilisation, while others think that they are different; for reference see Bard (1941), Best and Taylor (1945). It appears that both these schools of thought are correct.

Similarly there are two non-glycolytic anaerobic mechanisms, one of which is inhibited by oxygen and the other not, as shown by the fact that introduction of oxygen after asphyxiation may at first depress the response

or increase it from the outset. As the muscle responds if poisoned with iodoacetic acid in the presence of oxygen, one of these anaerobic mechanisms must be the same as the aerobic one.

Action of tone producing substances.—Tone producing substances relieve asphyxial arrest (Singh and Singh, 1948*a*). They activate the glycolytic as well as the non-glycolytic mechanisms, as shown by the following experiments. They do so better if the muscle is exhausted and their action is antagonised by glucose (Fig. 11). They may fail to have any

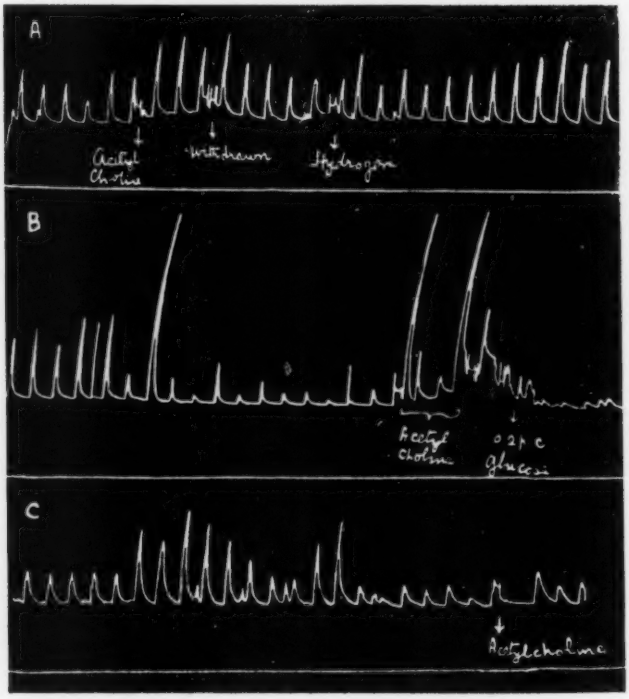

FIG. 11. Frog's stomach muscle.

A. Effect of acetylcholine in the presence of oxygen.

B. Same muscle as in A. Effect of acetylcholine in the absence of oxygen.

C. Same muscle as in A and B. The inhibitory effect of glucose on the action of acetylcholine.

effect in alkaline solutions and their effect increases with acidity (experimental range pH 8·5 to 6, Fig. 12). In the above instance, therefore, they activate the non-glycolytic mechanism. In alkaline solutions they usually

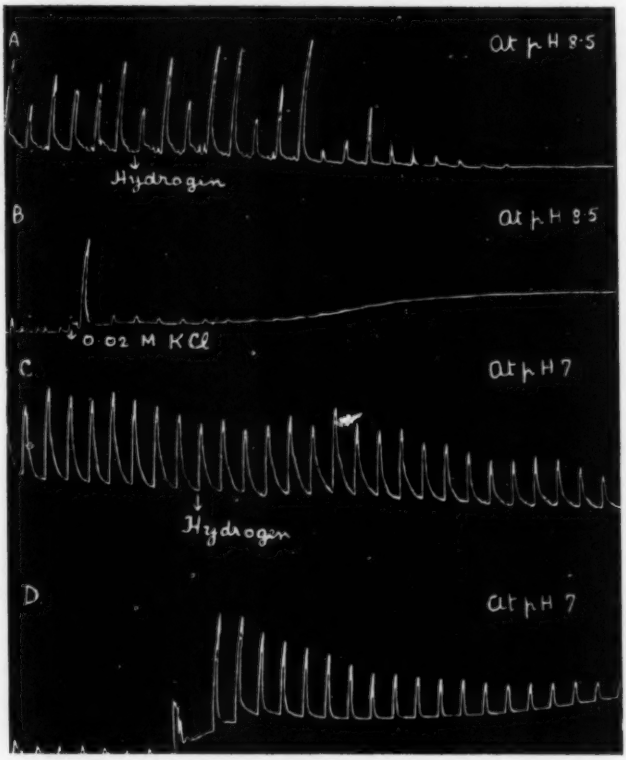

FIG. 12. Frog's stomach muscle. The effect of pH on the action of potassium on muscle paralysed by asphyxia.

activate the glycolytic mechanism as shown by the fact that iodoacetic acid suppresses their action, and glucose promotes it.

If the muscle is stimulated less frequently, once in 10–15 minutes instead of once a minute then the effect of tone producing substances is less marked as the muscle is less exhausted and may exactly be the opposite, that is, they inhibit the response (Singh and Singh, 1948*b*). In the presence of oxygen, potassium at first potentiates the response, which is followed by a secondary depressant action. In the brain, potassium stimulates the production of lactic acid in the presence of oxygen and suppresses it in asphyxia (Best and Taylor, 1945).

Action of acid solutions.—When the muscle is asphyxiated in acid solutions, the response may gradually decline. The anaerobic and the aerobic mechanisms are the same in these observations. If asphyxial

increase of excitability occurs, then the introduction of oxygen may be followed by depression; the aerobic and the anærobic mechanisms are, therefore, antagonistic.

Interaction between excitation and inhibition.—During the asphyxial increase in excitability, the inhibition produced by electrical current is diminished or replaced by contraction (Fig. 13). This occurs in both acid and alkaline solutions. This shows that the anærobic mechanisms for contraction and inhibition are antagonistic. Introduction of oxygen may suppress inhibition; this shows that the ærobic and anærobic mechanisms for inhibition are antagonistic.

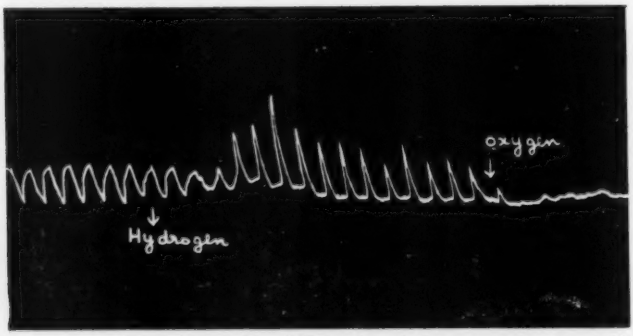

FIG. 13. Dog's stomach muscle. Effect of asphyxia on electrical inhibition.

An interesting effect of asphyxia is to produce " extrasystoles " (Fig. 14),

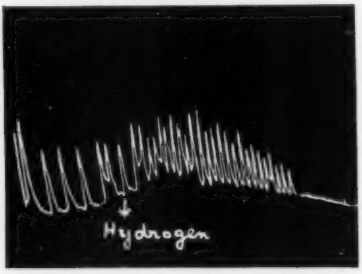

FIG 14. Fowl's gut. Extrasystoles produced by asphyxia.

as happens in the heart muscle as a result of coronary thrombosis. An inexcitable muscle may be rendered excitable by asphyxia.

DISCUSSION

Unstriated muscle appears to be a very complex tissue with interplay of metabolic mechanism also of great complexity. The metabolic mechanisms so far analysed are as follows:—

Anaerobic mechanisms.—There is a glycolytic and a non-glycolytic system. The former is of two kinds, one which gets rapidly exhausted and the other does not; again there are different glycolytic systems for tone and twitch respectively, as shown by increase of tone and suppression of the response to alternating current on addition of glucose (Singh, 1949).

Aerobic mechanisms.—There is a glycolytic and a non-glycolytic system. Some of these are similar to or identical with the anaerobic mechanisms described above. Each of these are divided one for tone and the other for twitch respectively. There is a system for supplying energy in acid solutions.

Summary

1. One kind of asphyxial increase in excitability is inhibited by glucose and increased by iodoacetic acid and acid solutions.

2. The second kind of asphyxial increase in excitability is increased by glucose and inhibited by iodoacetic acid and acid solutions.

3. The mechanism, which produces the glycolytic asphyxial increase in excitability is antagonistic to the non-glycolytic one.

4. Glucose is utilised anaerobically in two ways; one of these is the same as that in which it is utilised ærobically, and the other is antagonistic.

5. Tone producing substances depress the response of asphyxiated muscle to alternating current if the latter is stimulated about once in 10–15 minutes.

6. There are two anaerobic mechanisms in acid solutions; one is antagonistic to and the other same as aerobic one.

7. During asphyxial increase in excitability, inhibition may be turned into contraction.

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STUDIES ON THE EMBRYOLOGY OF MICROCHIROPTERA

Part IV. An Analysis of Implantation and Early Development in *Scotophilous wroughtoni* (Thomas)

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1. Introduction

The object of the present paper is to describe the early stages of development of the embryo and to elucidate the histological changes in the uterus during

this period. The earliest stage in my collection is a free uterine morula. I have not been able to collect earlier stages than this (probably I possess these stages in an allied species—*Scotophilus kuhli*—collected from Central Provinces, and I shall describe these stages in a later paper). From this stage onwards I have described the different stages progressively leading to implantation of the blastocyst and the early appearance of the fœtal membranes.

2. Historical

Though a large number of papers have been published relating to the embryology and the fœtal membranes of the insectivorous bats, details regarding certain important aspects of early development and particularly the stages preceding implantation are described by very few authors. Out of the fifteen families of Microchiroptera the embryology of only few families such as Rhinolophidæ, Hipposideridæ, Phyllostomatidæ, Molossidæ, and Vespertilionidæ has been described. No data is available regarding the other families. But even these existing accounts do not describe all stages of development and the process of implantation has not been worked out except in *Vespertilio murinus* (Van Beneden, 1888 and 1899; Duval, 1895; Nolf, 1896), *Nyctalus noctula* (van der Stricht, 1899 and 1911), *Miniopterus schreibersii* (Da Costa, 1920), *Vesperugo leisleri* (Ramaswamy, 1933), and *Myotis lucifugus lucifugus* (Wimsatt, 1944).

Van Beneden, during the last decade of the nineteenth century, published a number of papers on the early development and placentation of *Vespertilio murinus*, in which implantation is superficial. Duval's work (1894–1896) presents a detailed account of the early stages of development. Van der Stricht (1899–1911) recorded the curious fact in *Nyctalus noctula* that the primitive amniotic cavity was represented by a number of intercellular cleft-like spaces in the embryonic mass, and these, according to him, disappeared and the definitive amnion was formed later by folds. Da Costa (1919, 1920, 1922) worked, in fairly great detail, the early embryology of *Miniopterus schreibersii*. According to him the primitive amnion disappears and hence for some time the amniotic cavity is roofed over by the trophoblast. Da Costa's (1920) paper is important because of the excellent discussion on amniogenesis in mammals in general and Chiroptera in particular. Gérard (1928) gives a fairly detailed description of the allantochorionic placenta of *Vesperugo noctula*, but the early stages of development he does not describe. Sansom (1932) described some blastocysts of *Molossus rufus* and *Molossus obscurus*. In these specimens, unlike the other Chiroptera, there is a lateral orientation of the embryonic disc before placentation. Even in the other mammals such an orientation of the embryonic disc is rare, and

has been described only in *Chrysochloris* (De Lange, 1919). Ramaswamy (1932) described amniogenesis in *Vesperugo leisleri* by cavitation. Hamlett (1934) dealt with two species of South American bats—*Glossophaga soricina* (Phyllostomatidæ), and *Molossus crassicaudatus* (Molossidæ); but he also does not describe the early stages of development. In his later paper (1935) on *Glossophaga soricina* he gives details of placentation. Mossman (1937) describing the development of *Nyctinomus cynocephala* (Molossidæ) records the orientation of the embryonic disc as antimesometrial, first attachment circumferential, and implantation superficial. Wislocki and Fawcett (1941) working on *Artebeus jamaicensis* (Phyllostomatidæ) describe a simplex uterus as in *Glossophaga soricina* Hamlett (1934). They record the formation of a complete decidua capsularis, a rare phenomenon among the Microchiroptera. Wimsatt (1944 and 1945) working on *Myotis lucifugus lucifugus* (Vespertilionidæ) gives a detailed description of almost all the stages of early development and placentation. The orientation of the embryonic disc and the point of first attachment are antimesometrial. He describes the formation of the primitive amniotic cavity by cavitation, but the definitive amnion is formed by the closure of ectodermal folds. He thus confirms the earlier view of Nolf (1896) and Levi (1922). Wimsatt's paper embodies a very exhaustive description of the early development and placentation and I have tried to follow him in the general arrangement of this paper as far as possible.

3. Material and Methods

The females were found to be invariably pregnant during the months of April, May and June in progressively advanced stages. The earliest stages in my collection were collected on the 22nd March and full term embryos were found during the first week of June. A large number of pregnant females were collected. The pregnant uterii were fixed in Bouin's fluid directly after dissection; but in cases of advanced pregnancies the uterii were punctured with a view to allowing the penetration of the fixative. The uterii were cut serially at 10 μ and stained in Ehrlich's hæmatoxylin or Delafield hæmatoxylin and counterstained with eosin.

4. Observations

(*a*) *Pre-implantation development of the embryo*

(i) *The free uterine morula* (Pl. XX, Fig. 1).—The earliest stage obtained is a free morula lying in the uterine lumen. This morula covers nine sections and has a maximum diameter of 86 μ. It is triangular in section with its apex towards the mesometrial side. The morula shows a faint distinction between peripheral cells and a central mass of cells. The cells

at the periphery are cubical and are arranged in a regular manner and the cells in the centre are spherical and show no such regular arrangement. The cells are in active stages of division.

(ii) *The early blastocysts.*—The first differentiation of the embryonal cell mass and the surrounding trophoblast is indicated by the formation of narrow intercellular clefts in the central mass. These clefts all occur towards the mesometrial side (Pl. XX, Fig. 2). The morula has not increased much in size, the shape of the morula has changed slightly and the angularities are rounded off.

At a slightly later stage the intercellular clefts have coalesced to form a narrow crescentic cavity towards the mesometrial part of the embryo, thereby clearly differentiating the peripheral layer of cells which go to form the trophoblast from the central mass of cells which constitute the embryonic mass (Pl. XX, Figs. 3 and 4, Pl. XXI, Fig. 5). The blastocyst in Fig. 4 is almost spherical in shape. The trophoblast is made up of cubical cells, some of which show mitotic division stages, and the cells of the embryonic mass are irregularly spheroidal in shape and active. The blastocyst occupies 12 sections and has a maximum diameter of 112 μ and the embryonic mass a diameter of 82 μ.

The next stage in my collection is a blastocyst which is slightly more enlarged and has a diameter of 134 μ. This occupies 14 sections. At this stage the blastocyst cavity has enlarged in extent and is lined by the cubical trophoblast. The differentiation into embryonic mass and trophoblast is complete. The inner cell mass measures 92 μ, (Pl. XXI, Fig. 6).

The blastocyst undergoes enormous enlargement before further differentiation occurs (Pl. XXI, Fig. 7). This increase is supposed to be due to two causes according to Wimsatt (1944). There is a progressive accumulation of the fluid in the blastocyst cavity, and secondly there is a rapid proliferation of cells of the trophoblast. The increase in the extent of the cavity is parallel with the progressive flattening of the embryonic cell mass. The blastocyst measures 192 μ. It is roughly triangular and the embryonic mass occurs as a biconcave disc abutted against the inner surface of the trophoblast on the antimesometrial side and the blastocyst surface on the antimesometrial side is extensive. There is not much change in the cytology of the cells of trophoblast, while the cells of the embryonic mass have not only become smaller but numerous. The embryonic mass shows a slight differentiation of the endoderm on the mesometrial surface the embryonal mass, for, here, the cells covering the surface of the embryonic cell mass are

slightly flattened and clearly discernible as the forerunners of the future endoderm.

The blastocyst undergoes further enlargement without much differentiation in the next stage. This occupies 27 sections and has maximum diameters of 252 μ. The trophoblast in the section (Pl. XXI, Fig. 8) has shrunk and is therefore zigzag. There is a further flattening of the embryonic disc and the endoderm cells are confined only to a very small area round about the embryonic disc. The blastocyst occupies almost the whole of the uterine lumen. The trophoblast on the antimesometrial side of the blastocyst, *i.e.*, the trophoblast overlying the embryonic disc, is in contact with the maternal endometrium since the uterine epithelium is lost. The blastocyst is not yet implanted and the trophoblast is still unilayered.

(*b*) *Changes in the uterus preceding implantation*

(i) *Changes during the free uterine morula stage.*—The changes in the uterus before implantation of the blastocyst concern mostly the uterine epithelium, while the glands and the endometrium undergo profound changes during and after implantation. At the stage when the embryo is in the stage of an unattached morula, the uterine epithelium shows great activity: the cells hypertrophy and divide. Thus the epithelium appears to be made up of two or three layers of cells due to their crowding. The height of the epithelium varies in the different regions of the uterus, being tallest on the antimesometrial side. The part of the uterine epithelium in juxtaposition to the morula is hypertrophied—probably as a direct physiological response to the presence of the morula (Pl. XX, Figs. 1 and 2). The uterine glands are lined by active epithelial cells. The glands are elongated, dilated and often branched. The lumen contains secretion.

The uterine endometrium also shows activity, and there appears to be a descending gradient of activity as we pass from the antimesometrial side towards the mesometrial side of the uterus. On the antimesometrial side the endometrium just below the uterine epithelium is highly proliferative and presents a loose texture because of the accumulation of tissue fluid in the intercellular spaces. This results in an extension of the antimesometrial part of the uterine endometrium. A second change noticed is the pushing of the uterine glands from the antimesometrial side towards the lateral sides. Hence the uterine glands become almost absent from the antimesometrial side and are confined to the lateral and the mesometrial sides (Pl. XX, Fig. 1). The vascularisation of the endometrium increases markedly. The sub-epithelial region in particular is richly irrigated with blood and the capillaries form a network of vessels in this region.

(ii) *Changes during the early blastocyst stages.*—As development advances the changes seen in the previous stage are augmented and exaggerated. Parallel with the development of the embryo there is a rapid expansion of the uterine endometrium on the antimesometrial side resulting in a broadening of this part of the uterus. The uterine epithelium shows marked changes. In the region nearest to the surface of the blastocyst the hypertrophied epithelium shows signs of degeneration (Pl. XXI, Fig. 6). Fig. 7 shows the complete loss of the uterine epithelium on the antimesometrial side, and the degenerated cells occur as an eosinophilic cytoplasmic mass between the trophoblast and the uterine tissue on the antimesometrial side.

There is progressive loss of the uterine epithelium commencing from the antimesometrial part towards the mesometrial part. Finally when the unilaminar blastocyst has reached the maximum size the uterine epithelium is completely lost on all sides of the uterus (Pl. XXI, Fig. 8).

Due to the further expansion of the uterine endometrium in the antimesometrial region, the glands are further pushed down and finally come to be located only on the mesometrial side. The glands open widely into the uterine lumen (Pl. XXII, Fig. 9).

There is a further increase in the vascularisation of the endometrium, but most of the capillaries are situated on the antimesometrial side.

The changes in the uterus prior to implantation show clearly that the uterus can be distinguished into two physiologically distinct regions. The progestational changes are first manifest in the antimesometrial region and there is a regular retrogressive physiological gradient towards the mesometrial side. The changes in the endometrium are visible even before the embryo actually comes into contact with the uterine epithelium. The site of first attachment is defined even before actual attachment takes place, thereby indicating that the loss of the uterine epithelium is not due to the phagocytic activity of the trophoblast.

(c) *The process of implantation*

The phenomenon of implantation is the result of complementary changes occurring in the embryo and the uterus. Their cumulative effect is to attach the embryo to the uterus thereby facilitating physiological exchange between the fœtal and maternal tissues. At the time of implantation the embryonic mass is distinctly on the antimesometrial side, as is common in microchiroptera, the exceptions being two species of Molossid bats described by Sansom (1932) in which the orientation of the embryonic disc is lateral.

(i) *The early attachment of the blastocyst.*—The first indication of attachment is shown by the loss of the uterine epithelium in the antimesometrial part of the uterus. Pl. XXI, Figs. 6 and 7 indicate the very early stages in the loss of the uterine epithelium. The degenerative changes in the epithelium begin even before the embryo actually comes into contact with the uterus. Probably the loss of the uterine epithelium is due to overhypertrophy as a direct result of the uterine reaction to the presence of the embryo. Thus the phagocytic potentiality of the trophoblast can be accepted with some hesitation, and the capacity of the trophoblast as an enzyme secreting system to destroy the uterus requires further proof.

It is not till a later stage of nidation that the epithelium on the mesometrial side is also lost. The enormous distention of the endometrium on the anti-mesometrial side makes the implantation site free from glands.

The uterine endometrium likewise undergoes profound progestational changes and all these changes during the early stages of implantation concern the antimesometrial part. The endometriam can clearly be defined into two zones—a superficial zone (considering the uterine epithelium as the surface) of loose connective tissue made up of stellate or fusiform connective tissue cells, and a deeper zone of compact, fibrous connective tissue. The superficial zone is deciduous and is later invaded by the placental trophoblast, and the cells of this region are mostly lost by the activity of the trophoblast. The great vascularisation of this zone helps in easy physiological exchange between the maternal and the fœtal surfaces. The deeper compact zone is non-deciduous and is distinctly separated from the loose endometrial zone during later stages of gestation.

The uterine myometrium does not undergo many changes except those connected with the general swelling of the uterus.

(ii) *Further development of the blastocyst.*—The further development of the embryo consists, in addition to the general enlargement of the embryo, in the formation of the bilaminar omphalopleure and the amnion. The endodermal cells which were differentiated from the embryonal mass in the earlier stages migrate peripherally and come to underlie the inner surface of the trophoblast resulting in converting the unilaminar blastocyst into a bilaminar one (Pl. XXII, Fig. 12). The cells of the trophoblast are cubical and large, while the endodermal cells are fusiform.

Side by side with the differentiation of the endoderm the amniotic cavity is also formed. Its first indication is the occurrence of a small cleft-like space in the inner cell mass of the blastocyst which is just beginning to attach (Pl. XXII, Fig. 10). This cavity, the " primitive amniotic cavity,"

undergoes enlargement side by side with the flattening of the embryonal mass. The amniotic cavity increases in extent (Pl. XXII, Fig. 11) and is bound by the cells derived from the embryonal mass on all sides. On the antimesometrial side the roof of the amniotic cavity is reinforced by the trophoblast. The roof of the amniotic cavity, formed from the cells derived from the embryonal mass in the early stages of amniogenesis, continues as the definitive amnion. There have been different types of observation regarding the relationship of this layer of cells—" the primitive amnion "—with the definitive amnion. In *Scotophilus wroughtoni*, it is clear that the cells which form the roof of the " promitive amniotic cavity " continue as the definitive amnion. (Pl. XXII, Figs. 10, 11 and Pl. XXIII, Fig. 13). The amniotic cavity, therefore, is never continuous with the potential trophoblastic cavity, nor is it ever lined by the naked trophoblast, as has been described in some insectivorous bats by many authors.

Further development of the embryo consists in the establishment of the neural groove, the further differentiation of the amnion, and the formation of the chorio-allantoic placenta. I shall deal with these aspects in a separate paper.

(iii) *The late attachment of the blastocyst*.—At the time of actual attachment the blastocyst is completely bilaminar, and the uterine epithelium is completely lost. The trophoblast that overlies the embryonic mass is the first region to show implantation changes. As soon as this part of the trophoblast comes into cantact with the maternal tissue, it begins to proliferate. Thus this part of the trophoblast becomes multilayered. The placental trophoblast by a process of intensive proliferation begins actively to penetrate the uterine endometrium. Thus at first the trophoblast is seen to send cords of cells (Pl. XXIII, Fig. 13) which are invading the endometrium. But soon the cells of these chords become syncytial. Only the basal trophoblastic layer maintains the cell boundaries, and continues as the cytotrophoblast. The invading phagocytic, proliferative, placental trophoblast constitutes the syncytiotrophoblast. There is no definite boundary between the two regions of the trophoblast, one imperceptibly merging into the other.

The cells of the cytotrophoblast are large, cubical, and have distinct cell boundaries, the cytoplasm is granular and the nuclei are active. The nuclei of the syncytiotrophoblast are large and are also in active division. The invasion of the maternal endometrium which commences on the antimesometrial side gradually extends to the lateral sides. On the mesometrial side the trophoblast is not penetrative but remains a single layer of cells.

The penetrating syncytiotrophoblast progressively migrates inwards from the uterine epithelium towards the deeper layers of the endometrium. The endothelial lining of the maternal capillaries persists for a while, but later it is also lost and the maternal blood thus comes to lie in large lacunæ in the syncytiotrophoblast. The final placenta conforms to the labyrinthine hæmochorial type (Pl. XXIII, Fig. 14).

(*d*) *Fœtal membranes after implantation*

At the end of implantation, the embryo is made up of an embryonic disc showing a clear distinction into ectoderm underlying the amniotic cavity, —the amnion formed by a single layer of cells closely applied to the basal cytotrophoblast,—and the trophoblast. The embryonic disc shows a distinction into endoderm and ectoderm, the mesoderm has not yet been differentiated. The trophoblast itself can be distinguished into two regions: (i) the trophoblast at the embryonic pole, *i.e.*, the future placental site; (this trophoblast gives origin to the syncytiotrophoblast which invades the maternal tissues to establish the placenta,) and (ii) the trophoblast at the abembryonal pole which forms the outer layer of the bilaminar omphalopleure, the inner layer of the yolk-sac being formed by the thin endoderm. The cavity of the yolk-sac is large.

As in most of the microchiroptera there is no decidua capsularis.

5. Discussion

(*a*) *The orientation of the embryonic disc and the process of implantation*

Mossman (1937) considers that the orientation of the embryonic disc of the mammals is of some systematic significance. He states, "The orientation of the disc with reference to the uterus is usually quite constant in all groups known to be closely related." Most of the authors working on the embryology of Microchiroptera have shown that the embryonic disc is orientated anti-mesometrially from a very early stage of development. Only Sansom (1932) has recorded in two species of Molossidæ—*Molossus rufus* and *Molossus obscurus*—a lateral orientation of the embryonic disc in the blastocyst stage. This is a rare phenomenon amongst the mammals, the only other mammal showing lateral orientation of the disc is *Chrysochloris* (De Lange, 1919). In another species of Molossidæ—*Nyctonomus cynocephala*—Mossman (1937) has recorded an antimesometrial orientation of the embryonic disc. In *Scotophilus wroughtoni* even at the stage of the early blastocyst the embryonic knob is on the antimesometrial side, and this disposition of the embryonic disc becomes very obvious in later stages of development (Pl. XXI, Figs. 5 & 6). Probably the orientation of the

embryonic disc, the implantation of the embryo, and the mode of amnion formation are closely related phenomena, though a clear proof of this relationship has not been attempted.

As regards the phenomenon of implantation itself there are two contrasting views on the question of the factors responsible for bringing about the changes in the uterus. Some authors believe that the loss of the uterine epithelium is due to the activity of the trophoblast, which destroys the epithelium either by phagocytosis or by the secretion of a cytolytic enzyme. Another set of observers seek for the stimulus for effecting these changes in the mother herself, and they attribute the progestational changes to the activities of the endocrines.

That the embryo takes an active part in the destruction of the uterine epithelium by either phagocytosis or by the secretion of a cytolytic enzyme is upheld by Sansom and Hill (1931) in the Guinea pig, Heuser and Streeter (1941) in the Macaque monkey, and by Hertig and Rock (1941, 1944, 1945) in the human being. Working on the implantation of the golden hamster, *Cricetus auratus*, Margaret Ward (1948) observes, " The first changes appear to be caused by pressure; the disintegration appears to be due to cytologic activity of the conspicuously vacuolated trophoblast cells, as degeneration is especially obvious at either side of the processes from the cells. Although there is some phagocytosis, this appears to be definitely secondary to the cytolytic activity at this time ".

Positive proof in support of this view that the embryo is an active phagocytic agent has been furnished by Runner (1947) in the mouse. He observes that the mouse egg is capable of developing in the anterior chamber of the eye and that the embryo can implant inside the iris and get surrounded by hæmorrhage. He, therefore, concludes, " It would appear to lie within the power of the embryo to implant and thereby induce the decidual reactions (in uteri which have appropriate thresholds of reactivity) " (page 9).

Wislocki and Streeter (1938) working on the placentation of the Macaque monkey (*Macaca mulatta*) believe that the decidual reaction in the maternal endometrium is induced by the ovum, and probably the stimulus is of a chemical nature.

Quite a large number of observers on the other hand disbelieve in the activity of the embryo and find the cause for effecting the uterine preimplantation changes inside the mother herself. Klein (1934) in the rabbit, and Megliavacca (1935) in the rat have shown that there is epithelial degeneration in the sterile horn in unilateral pregnancy. In the ovariectomised Rhesus monkey, in the pre-secretory corporin phase in the experimental

cycles, Hisaw (1935) produced progestational changes resulting in hypertrophy and degeneration of the uterine epithelium by the injection of ovarian harmones.

Mossman (1937) believes that the uterine changes in the pre-implantation stages are regulated by endocrinal factors in the mother and these are quite independent of the ovum. Krehbiel (1937, 1941) supports this view. He produced, in the rat, progestational changes in the sterile horn in unilateral pregnancy and, in lactating rats by the application of electrical and mechanical stimuli. Both Mossman (1937) and Krehbiel (1937) believe that the breakdown of the epithelium is due to the localised anæmic condition caused in the implantation site caused by an interference with blood supply due to edema formations, and the embryo does not play a direct role in the removal of the uterine epithelium. Krehbiel states that a disrupted vascular supply leads to the lifting of the epithelium, and that fatty degeneration then overtakes the undernourished cells, and any damage the embryo causes is due to the pressure exerted by its growth.

Wimsatt (1944) working on *Myotis lucifugus lucifugus* states that the first breakdown of the uterine epithelium is mainly due to the internal causes confined to the mother and only the ultimate elimination of the epithelial elements is due to the phagocytic activity of the trophoblast. There is no cytolising action on the maternal epithelium at this stage, though he admits that during the later stages cytolysis may play a role in the destruction and resorption of the connective tissue and blood vessels.

That the ovum has no part to play in the early progestational changes has also been demonstrated by Blandau (1947) and Alden (1947) who have produced these changes by the use of artificial " ova " of glass or paraffin. The uterine changes observed during pseudopregnancy in certain mammals which are similar to the changes during normal pregnancy also confirm this view.

Working on the rat, Alden (1948) has concluded that the preliminary degenerations are brought about by factors within the mother, while the final destruction of the epithelium is caused by the phagocytic activity of the trophoblast. He states " It seems possible that endocrine (and possibly vaso-motor) stimuli reach the uterus and effect significant changes in the vascular supply....and that due to this the surface epithelium becomes altered and may indeed be loosened from its basement membrane...,during and following these events the embryo does not remain passive, but through its specialised extraembryonic tissue brings about the final destruction, dissolution and phagocytosis of the epithelium ".

(*b*) *The loss of the uterine epithelium*

In *Scotophylus wroughtoni* the uterine epithelium on the antomesometrial side of the uterus at as early a stage as the early blastocyst shows hypertrophy and is lifted up from the endometrium (Pl. XXI, Fig. 6), and at a slightly later stage the epithelium is completely lost (Pl. XXI, Fig. 7). The blastocyst is still small and quite at a distance from the uterine surface. The phagocytic activity of the trophoblast cannot be accepted as a primary stimulus for the loss of the uterine epithelium. It is only later that the degenerating epithelial debris is seen near the trophoblast. The theory of Mossman and Krehbiel that the loss of epithelium is due to an anæmic condition caused by a congestion of blood resulting in an undernourishment of the epithelial cells cannot be accepted because, in the case of *Scotophylus wroughtoni*, during these stages, when the uterus is undergoing changes, the vascularisation of the sub-epithelial region is definitely great. Whatever secondary rôle the embryo may play, by far the most important stimulus for the initiation of the uterine changes comes from factors within the female herself. The potentiality of the trophoblast as an organ secreting cytolytic enzyme is still debatable and requires further proof. I have not been able to confirm the observation of Wimsatt (1944) that there is a thinning of the uterine epithelium before actual destruction. On the other hand, there is clear evidence to show that the hypertrophied epithelium gets ruptured from the uterine endometrium due to the profound endometrial changes, and also due to its enormous extension. So the subsequent removal of the degenerating cells by the trophoblast is a service rendered by the embryo for effective nidation. But during later stages of nidation when the trophoblast is actively penetrating the endometrium the trophoblast definitely acts phagocytically and removes the endometrial tissue which has become loose due to hypertrophy.

(*c*) *Amniogenesis*

An excellent review of the modes of amnion formation is given by Da Costa (1920). Even in a small group as the Microchiroptera three different types of observations have been recorded:—

(i) The amniotic cavity arises as intercellular clefts in the embryonic knob and these clefts coalesce to form a large cavity—the primitive amniotic cavity. This is at first roofed over by the cells of the embryonic knob itself and this roof constitutes the primitive amnion. But as the primitive amniotic cavity expands, the primitive amnion is lost so that the amniotic cavity is contiguous with the potential trophoblastic cavity, and is, therefore, roofed over by the trophoblast. The definitive amnion is later formed by the upgrowth of folds from the margin of the embryonic disc, which

gradually underlie the trophoblast. Such a method of amniogenesis has been described by van Beneden (1888 and 1889) in *Vespertilio murinus*, Da Costa (1920) in *Miniopterus schreibersii*, Sansom (1932) in *Molossus rufus* and *Molossus obscurus*, Ramaswamy (1932) in *Vesperugo leisleri*, Hamlett (1934) in Rhinolophidæ, Vespertilionidæ and Molossidæ, and Wimsatt (1944) in *Myotis lucifugus lucifugus*.

(ii) The amniotic cavity arises as in (i), or by a rearrangement of cells of the embryonic knob, and this cavity is roofed over by the cells of the embryonic knob alone. This roof is at first in contact with the trophoblast, but as development proceeds it is separated from the trophoblast. The definitive amnion is the same layer of cells which roofed over the amniotic cavity when it first formed; it only undergoes separation during later stages of development. There is thus no distinction between the primitive amnion and the definitive amnion. This method of amnion formation has been described by Hamlett (1934 and 1935) in *Glossophaga soricina* (Phyllostomatidæ).

(iii) The amnion in *Nyctinomus cynocephala* (Molossidæ) has been described by Mossman (1937) to arise by " Folding ".

van der Stricht (1899 and 1911) describes a curious phenomenon in *Nyctalus noctula* where he affirms the origin of the primitive amniotic cavity as a number of intercellular clefts, but he states that all these spaces do not get incorporated in the amniotic cavity. In fact he described the disappearance of these spaces, and the formation of the definitive amnion later.

In *Scotophylus wroughtoni* even at the earliest stage when the amniotic cavity is still very small, the cavity is single and appears to arise by a rearrangement of cells of the embryonic knob. The cavity is roofed over by the cells of the embryonic knob and this roof is in contact with the trophoblast. As development proceeds there are two changes occurring to this roof; first of all a stretching of the roof due to a widening of the amniotic cavity, and secondly a separation of the roof from the trophoblast. Thus the primary lining which was two or three cell thick, becomes thin and forms a single layer of cells, but does not suffer destruction, as happens in *Myotis lucifugus lucifugus* (Wimsatt, 1944). No distinction between the primitive amnion and the definitive amnion can be made out, they being two different stages during the development of the same structure. The two terms are synonymous, at least in the case of *Scotophilus wroughtoni*. The establishment of the amniotic cavity in the embryonic knob has also resulted in the establishment of the definitive amnion. The amniotic cavity is, therefore, never, at any time, roofed over by the naked trophoblast, and no ectodermal

fold formation ever occurs. This mode of amnion formation is similar to that described by Hamlett in *Glossophaga soricina* (Hamlett, 1934 and 1935).

(*c*) *The fate of the uterine glands*

There are two views put forth regarding the loss of the uterine glands from the antimesometrial side during the process of implantation. van Beneden, van der Stricht and Wimsatt have recorded that these glands do not take any active part in placental formations. Wimsatt has shown that these glands are mechanically pushed laterally, and finally mesometrially due to the differential expansion of the endometrium of the antimesometrial part of the uterus, but Nolf (1896) believed that the uterine glands undergo resorption and get lost during placentation. In *Scotophilus wroughtoni* my observations lead me to support the first view that the glands are pushed towards the mesometrial side due to hypertrophy and changes in the uterine endometrium on the antimesometrial part (Pl. XXII, Fig. 9).

6. Summary

1. From the very early stages of development the orientation of the embryonic knob is antimesometrial.

2. The site of implantation is clearly defined antimesometrially even before the embryo is actually attached.

3. The loss of the uterine epithelium cannot be attributed to the phagocytic activity of the trophoblast, though the trophoblast may ingest the degenerated epithelial cell dêbris. Only later the trophoblast is seen to actually erode the uterine endometrium for estblishing placental relationships.

4. Amniogenesis occurs by cavitation. No distinction into primitive and definitive amnion can be made out, because they are one and the same; the definitive amniotic cavity is differentiated when the amniotic cavity is formed.

5. The uterine glands do not undergo resorption, but are mechanically pushed towards the mesometrial side due to the widening of the antimesometrial part of the uterus.

7. Acknowledgement

I am very grateful to Prof. M. A. Moghe, College of Science, Nagpur, for his valuable guidance throughout this investigation and his kind help in placing his personal library at my disposal.

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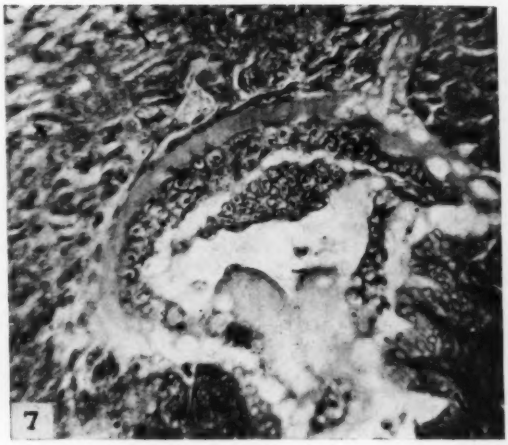

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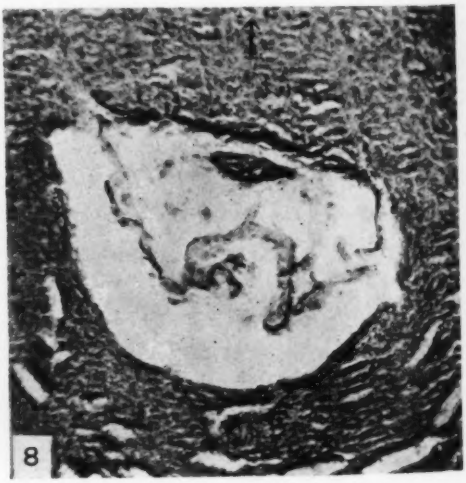

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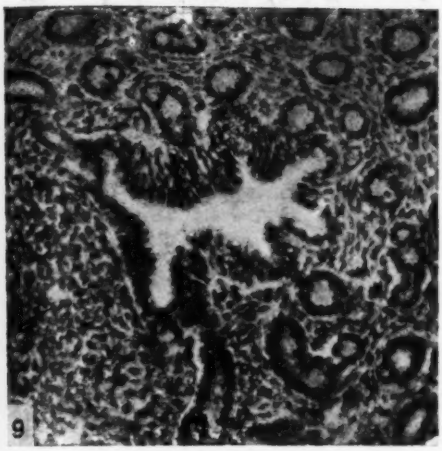

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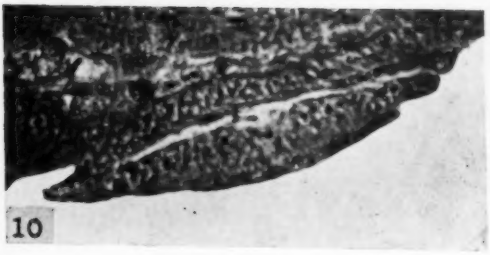

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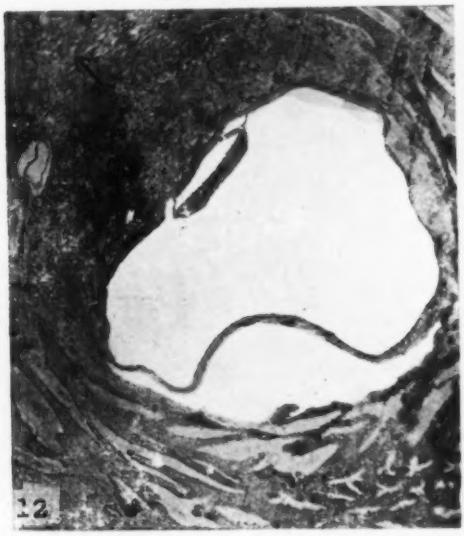

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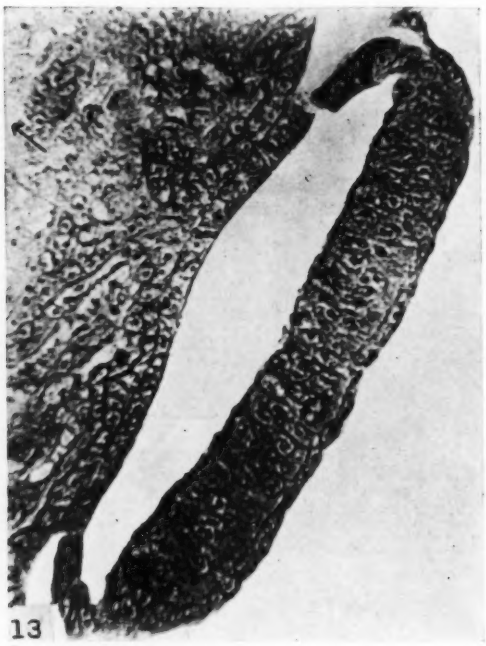

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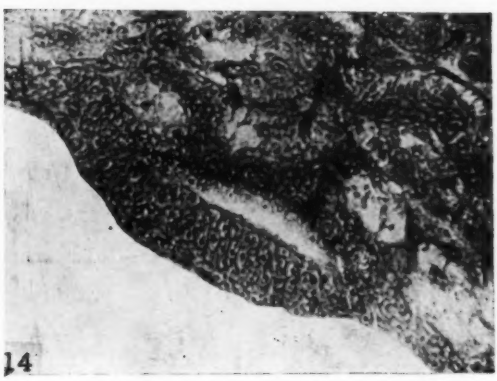

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9. Explanation of Plates

(The arrow points to the anti-mesometrial side except in Fig. 1)

Fig. 1. Section of the uterus with a free uterine morula. The uterine epithelium is intact on all sides. Note the complete absence of the uterine glands from the anti-mesometrial side. The uterine endometrium on the antime sometrial side is becoming loose. (Arrow points mesometrically.)

FIG. 2. Free uterine morula. Some of the cells are showing metaphase plates. The uterine epithelium is intact on all sides. The morula shows the beginning of intercellular cleft.

FIG. 3. Early blastocyst with the blastocyst cavity formed, and the inner cell mass differentiated. The uterine epithelium is hypertrophied but is intact on all sides.

FIG. 4. The blastocyst at a later stage than the one in Pl. XX, Fig. 3. The blastocyst cavity occurs as a crescentic cleft.

The inner cell mass is large and is attached to the inner surface of the trophoblast on the antimesometrial side. The uterine epithelium is intact.

FIG. 5. The blastocyst cavity has slightly increased in extent than in Fig. 4, the uterine glands are absent from the antimesometrial side of the uterus. Note that on one side the uterine epithelium is lifted up from the endometrium.

FIG. 6. Blastocyst is more advanced in development than in Pl. XXI, Fig. 5. The cavity of the blastocyst has further increased in extent. The epithelium of the uterus has greatly hypertrophied and on one side it is markedly lifted from the endometrium.

FIG. 7. An advanced stage of a unilaminar blastocyst. The inner cell mass is showing the early beginnings of the formation of the endoderm, which extends only up to the margin of the embryonic disc. The uterine epithelium on the antimesometrial side is completely lost, and the epithelial débris occurs as eosinophilic cytoplasmic mass between the trophoblast and the endometrium on the antimesometrial side. The epithelial lining is, however, preserved on the other sides of the uterus. The endometrium on the antimesometrial side is loose.

FIG. 8. A late unilaminar blastocyst which is attached to the uterine endometrium on the antimesometrial side. The trophoblast on the other sides is free and is lying zigzag due to shrinkage. The uterine epithelium is, however, lost from all sides The uterine glands are confined to the lateral and the mesometrial sides. This is best seen in the next figure.

FIG. 9. Section of the pregnant uterus showing the beginnings of the migration of the uterine glands towards the mesometrial side. Note the very highly proliferating uterine epithelium, which appears to be made up of two or three layers of cells due to overcrowding of cells. Note also the epithelium on the antimesometrial side being lifted from the endometrium.

FIG. 10. Section of a late blastocyst showing the early formation of the amniotic cavity which occurs as a slit, and is roofed over by the fusiform cells of the inner cell mass, and is reinforced by the basal trophoblast. Towards the left side of the figure note that the trophoblast shows the occurrence of cords, which are penetrating into the uterine endometrium.

FIG. 11. The blastocyst is at a later stage of development than Pl. XXII, Fig. 10. The amniotic cavity has increased in extent.

FIG. 12. A late bilaminar blastocyst showing the large amniotic cavity. The antimesometrial part of the uterus is showing decidual formation. The glands all occur towards the mesometrial side.

FIG. 13. Same as Pl. XXII, Fig. 12. The amnion is distinct from the basal trophoblast. The latter is actively sending cords into the endometrium. The endoderm is clearly made up of fusiform cells.

FIG. 14. A late blastocyst showing the clearly differentiated endoderm. The large spaces in the uterine endometrium are blood lacunæ surrounded by the syncytiotrophoblasts.

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PRELIMINARY STUDIES IN THE USE OF SYNTHETIC HORMONES AS WEED-KILLERS IN THE BOMBAY PROVINCE

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In recent years considerable interest has been aroused in a new weed-killing chemical and several articles have appeared in scientific as well as popular journals advocating the virtues of this chemical variously described as "2, 4-D" "M.C.P.A.", "D.C.P.", "Hormone", "Methoxone", etc. The earliest trials with these materials, which are of the nature of growth-regulating substances, as weed-killers were carried out in England and the United States. Such promising results were obtained that within three years of its introduction 2, 4-D and allied products have become the most widely used weed-killers in these countries. Harvey and Robbins (1947) and Kephart and Evans (1948) have published lists of weeds and crops susceptible or resistant to 2, 4-D in United States of America. Similar lists are also supplied by the manufacturing firms in other temperate countries. It has also been suggested by Van Overbeck (1947) that these chemicals would prove effective in controlling tropical weeds but no detailed lists of susceptible weeds in tropical countries are available.

These weedicides are now beginning to reach India and interested parties are anxious to know how far they will prove useful in controlling the weeds commonly found here. The Economic Botanist to Government, B. P., Poona, has been supplied with small quantities for experimental purposes by some firms interested in marketing these preparations in India, during the last two years. The purpose of this article is to summarize all the information that has been obtained during these preliminary trials.

Four different preparations were tried, *viz.*,

1. Methoxone or Agroxone containing 10% sodium-4-Chloro-2-Methyl-phenoxyacetate as the active principle, supplied by the Imperial Chemical Industries.

2. Fernoxone containing 80% of the sodium salt of 2, 4-Dichloro-phenoxyacetic acid also supplied by the Imperial Chemical Industries.

3. Weed-no-more containing 40% of the Butyl ester of 2, 4-dichlorophenoxyacetic acid supplied by Sherwin-Williams Co., Cleveland, U.S.A., through Thomas Cook & Son, Bombay.

4. Weedone containing 10% of an unstated compound of 2, 4-dichlorophenoxyacetic acid supplied by May & Baker, England.

These weed-killers differ entirely in their mode of action from all chemicals previously used as weedicides in that they are not poisonous or corrosive. They were first used in very low concentrations of 1 to 10 parts per million to stimulate the growth of plants. Higher concentration of 100 to 1,000 parts per million resulted in overstimulation and a short period of rapid abnormal growth followed later by death of certain plants. Different plants respond to the action of these chemicals in different ways. Some show a twisting and bending of the stems and leaves; others show a swelling of the tops of shoots or at the nodes, with cracks developing later; still others show only a drying of stems and leaves. All these responses have been observed in the preliminary trials herein reported (Fig. 1).

Different species of weeds as well as cultivated crops show wide differences in their response to the new herbicides. Some plants are killed by very small quantities; others do not show any signs of injury when much larger doses are used. This differential response of various plants to these chemicals places them in the class of selective weed-killers. All grasses and cereals like wheat, barley, rice, *sorghum* and maize are not affected in the least. Linseed, strawberries, tea and coffee plants are likewise resistant. These weedicides can, therefore, be used in the crops mentioned above for eradicating a number of weeds without fear of harming the crops. On the other hand, crops like cotton, tobacco, tomato, sweet-potato, brinjal, chillies and all legumes are extremely susceptible. Therefore, great care must be taken to see that sprays and dusts of these chemicals do not drift on to susceptible crops in fields nearby.

With this background, we may proceed to describe the results obtained in our preliminary trials with the preparations supplied by various firms. Of these, except Fernoxone which was in powder form, the rest were supplied in liquid form. They were applied in the form of a spray. The concentrations used were 0·05%, 0·1% and 0·2% of the active ingredients. If applied at the rate of 100 gallons per acre this amounted to the application of ½ lb., 1 lb., and 2 lbs. respectively of the active constituents.

As a result of these tests which have been carried out on both actively growing and mature herbaceous weeds of each of the species mentioned, it has been possible to classify the weeds into three classes, *viz.*,

1. Susceptible, *i.e.*, those which succumb to applications of ½ to 1 lb. per acre.

2. Intermediate, *i.e.*, those which require applications of at least 2 lbs. per acre for obtaining a kill.

3. Resistant, *i.e.*, those which show no signs of injury or recover in spite of injury even when treated at the rate of 2 lbs. per acre.

The accompanying tabular statement shows the degree of susceptibility of common weeds, their response and the end result of spraying with the new weed-killers. It must be emphasized that the classification is tentative and liable to change as more tests are conducted and more information becomes available. The response of plants is conditioned by several factors of which the stage of growth of the plant and the prevailing temperature are the most important. It must also be pointed out that we have noticed no particular differences in the effectiveness of the different preparations used. Therefore, when these products are offered for sale, it would be necessary to compare the prices on the basis of active constituents contained in each product before making a final choice.

There are two weeds in the above test, which are considered very serious pests in the Bombay Province, *viz.*, *Cyperus rotundus* (*lavala*) and *Striga* sp. It is, therefore, necessary to discuss the effect of 2, 4-D products on these weeds in greater detail.

Cyperus rotundus.—Though very closely related to plants belonging to the *Gramineæ* (which are resistant to these chemicals), *Cyperus rotundus* was badly injured by a 0·2% solution of these weedicides. The treated plants showed no response till the 6th day after treatment; thereafter, there occurred a sudden drying and drooping of the leaves which resulted in the death of the plants in 10-15 days (Figs. 2 & 3). Underground tubers from treated and killed plants when planted in pots and provided with favourable conditions for germination, failed to sprout, thereby proving that the chemical had been translocated downwards. Underground shoots at depths of six inches were also killed. A critical study on the control of this obnoxious weed is underway. The preliminary trials lead us to believe that the use of these new weedicides will afford an effective method of controlling *Cyperus rotundus*, in crops like wheat, *Sorghum* and in lawns.

Striga lutea and *S. densiflora*.—The weeds belong to the *Scrophulariaceæ* and are very common phanerogamic root-parasites on *Sorghum* in the Bombay Province. So far, clean cultivation and weeding the *Striga* plants before they flower is the remedy usually suggested. In our trials, both species of

Table showing the degree of susceptibility of common weeds, their response and the end result of spraying with the new weedicides

S. No.	Name of plant	Degree of susceptibility	Response	End result of spraying
1	*Acalypha indica* Linn	Susceptible	Bending and swelling of stems	Died in 16 days
2	*Ageratum conyzoides* Linn.	Intermediate	Bending of shoots and drying of leaves	Died in 20 days
3	*Alysicarpus rugosus* DC.	Resistant	Unaffected	..
4	*Amarantus spinosus* Linn.	Susceptible	Bending of stems and yellowing of leaves	Died in 18 days
5	*Argemone mexicana* Linn.	Susceptible	Bending and parching of leaves	Died in 12 days
6	*Bidens pilosa* Linn. ..	Susceptible	Bending and swelling of stems	Died in 16 days
7	*Boerhaavia diffusa* Linn.	Intermediate	Twisting of shoots & yellowing of leaves	Died in 20 days
8	*Cassia tora* Linn.	Resistant	Epinasty of shoots	Plants recovered
9	*Cæsulia axillaris* Roxb.	Susceptible	Bending and swelling of stems; yellowing of leaves	Died in 15 days
10	*Celosia argentea* Linn.	Susceptible	Swelling at nodes which burst later	Died in 16 days
11	*Commelina benghalensis* Linn.	Intermediate	Swelling at nodes and yellowing of leaves	Died in 20 days
12	*Cyperus rotundus* Linn.	Intermediate	Withering of foliage	Died in 15 days
13	*Corchorus trilocularis* Linn.	Susceptible	Bending and swelling of stems, drooping of leaves	Died in 18 days
14	*Datura fastuosa* Linn.	Susceptible	Drooping of leaves, bending and swelling of shoots	Died in 14 days
15	*Datura stramonium* Linn.	Susceptible	do	Died in 14 days
16	*Eclipta alba* Clke.	Susceptible	do	Died in 16 days
17	*Euphorbia geniculata* Ort.	Susceptible	Bending of shoots which crack later	Died in 18 days
18	*Euphorbia thymifolia* Linn.	Susceptible	Parching of leaves	Died in 12 days
19	*Euphorbia pilulifera* Linn.	Intermediate	Twisting of shoots and drooping of leaves	Died in 20 days
20	*Fimbristylis ferruginea* Vahl.	Resistant	Withering of above water foliage	Recovers
21	*Indigofera glandulosa* Willd.	Susceptible	Bending and swelling of tops; yellowing of leaves	Died in 18 days
22	*Ipomœa muricata* Jacq.	Susceptible	Chlorosis of leaves; withering of plant	Died in 12 days
23	*Ipomœa reniformis* Chois.	Resistant	Unaffected	..
24	*Lagasca mollis* Cav.	Susceptible	Swelling of shoot tips which rupture later	Died in 14 days
25	*Leucas aspera* Spr.	Susceptible	Epinasty and chlorosis of leaves	Died in 12 days
26	*Leucas stricta* Bth.	Susceptible	Curvatures of shoots and chlorosis of leaves	Died in 12 days
27	*Mirabilis jalapa* Linn.	Intermediate	Bending of shoots and drooping of leaves	Died in 20 days
28	*Mimosa pudica* Linn.	Resistant	Unaffected	..
29	*Nicandra physaloides* Gaert.	Susceptible	Bending of stem and drying of leaves	Died in 18 days
30	*Ocimum canum* Sims.	Susceptible	Drooping and drying of leaves	Died in 15 days
31	*Oldenlandia corymbosa* Linn.	Resistant	Unaffected	..
32	*Oxalis corniculata* Linn.	Resistant	Unaffected	..
33	*Phyllanthus urinaria* Linn.	Susceptible	Bending and swelling of stems	Died in 12 days
34	*Portulaca oleracea* Linn.	Susceptible	Yellowing and drying of shoots	Died in 18 days

TABLE—*Contd.*

S. No.	Name of plant	Degree of susceptibility	Response	End result of spraying
35	*Psoralea corylifolia* Linn.	Susceptible	Bending, swelling and cracking of shoots; chlorosis of leaves	Died in 18 days
36	*Sesuvium portulacastrum* Linn.	Intermediate	Bending and swelling of shoots	Died in 20 days
37	*Sonchus arvensis* Linn.	Susceptible	Twisting of shoots, chlorosis of leaves	Died in 15 days
38	*Sonchus oleraceous* Linn.	Susceptible	Bending and swelling of shoots; chlorosis of leaves	Died in 15 days
39	*Solanum nigrum* Linn.	Susceptible	Chlorosis of leaves and drying of shoots	Died in 15 days
40	*Striga densiflora* Bth.	Susceptible	Curvature of shoots and leaves which wither later	Died in 10 days
41	*Striga lutea* Lour.	Susceptible	do	Died in 10 days
42	*Tridax procumbens* Linn.	Susceptible	Epinasty, chlorosis and drying of leaves	Died in 15 days
43	*Xanthium strumarium* Linn.	Susceptible	Parching of leaves ond shoots	Died in 15 days

Striga have responded very quickly to the application of 0·1% of these weedicides. Twenty-four hours after treatment, the plants show distinct curvatures of shoots and shrivelling of leaves (Fig. 4). The plants were completely killed within a week of spraying while the host plant was unaffected. So destruction of these parasites after they appear above the surface of the soil could be speedily and efficiently accomplished by the use of these weedicides.

But these root-parasites complete nearly half of their life-history underground (*i.e.*, 4-6 weeks) during which time they are feeding on the roots of the host. By the time they appear above ground they have done a good deal of damage and even if they are removed, there may be others which continue to feed on the host. It was, therefore, considered worthwhile to study the effect of these herbicides on the parasites before they emerged above the surface of the soil. For this purpose, wet blotting papers with seeds of *Striga* strewn on them were inserted and kept close to the inner walls of glass jars, so that the *Striga* seeds were easily seen from the outside. The glass jars were filled with soil and seeds of a susceptible variety of *Sorghum* were sown in them. After about five weeks when the maximum number of haustorial connections had been formed by the *Striga* seedlings with the host, the soil surface in each glass jar was treated with a measured quantity of 0·1% of the weedicides. Detailed observations taken after treatment go to show, that the underground *Striga* seedlings present upto a depth of

six inches were killed in about six days time. From these trials it would, therefore, appear that these weedicides are capable of destroying not only the above ground parts of *Striga* plants but the underground seedlings as well. We are hopeful that these findings may point the way to controlling the depredations of *Striga* which may be both effective and within the means of the cultivator. *Striga* attack on *Sorghum* is a problem of longstanding importance in the Provinces of Bombay, Central Provinces and Madras and it is quite likely that the use of these weedicides may effectively solve this problem. The control of *Striga* by these means on a field scale is being studied in all the detail and seriousness the problem demands.

The above are two weed problems confronting the agriculturists in the Bombay Province. The new hormonal weedicides are likely to help considerably in tackling them. As regards how far the new products may be utilised in controlling the other weeds listed in the table, it is difficult to say. This would depend upon the intensity of the infestation and the cost of the new method of control as against the old one. Each problem deserves to be studied from all aspects. It is hoped that this preliminary report may result in a more extensive trial of synthetic hormones of controlling tropical weeds and furnish data which is sure to come in handy whenever or wherever any particular weed becomes a serious pest.

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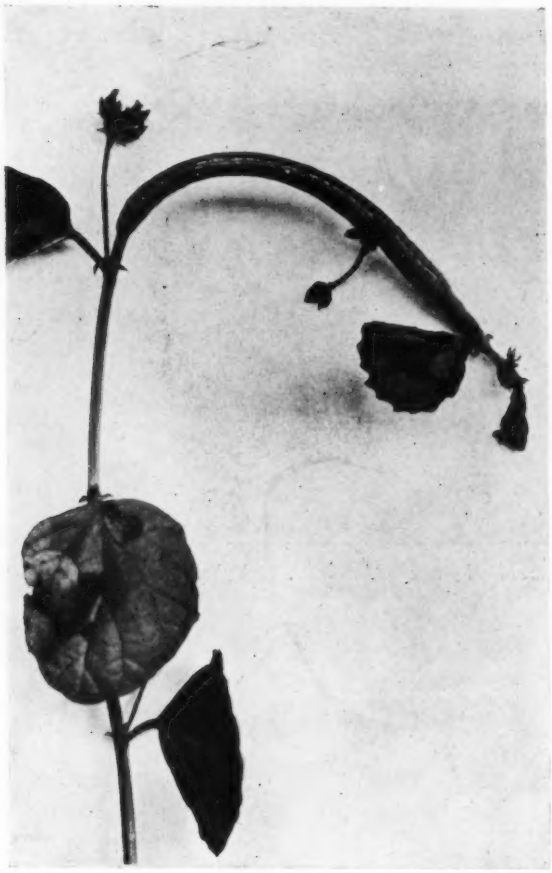

FIG. 1. *Psoralea corylifolia*, a dicot weed showing bending, swelling and cracking of the shoot as a result of spraying with 2, 4-D.

FIG. 2. *Cyperus rotundus* ('*Lavala*') plants before treatment.

FIG. 3. The same set of plants as in Fig 2, 11 days after spraying with 0·2% Agroxone.

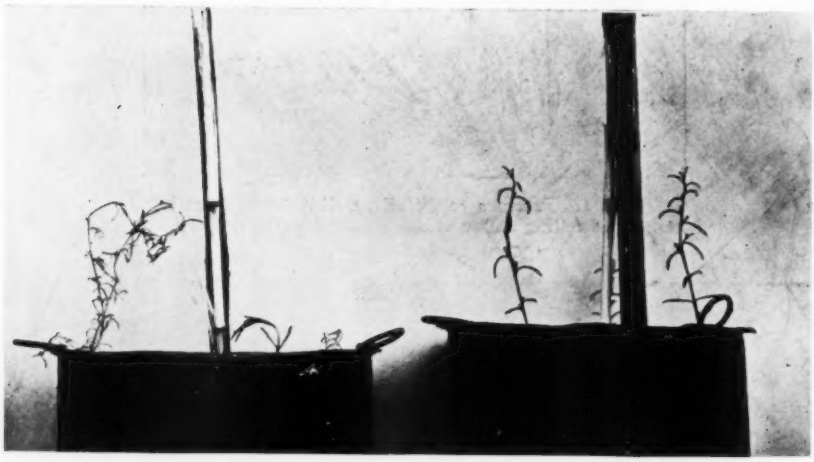

FIG. 4. On the left—*Striga* showing epinasty and shrivelling up of leaves, 24 hours after spraying with 0·1% Agroxone. Unsprayed plants on the right.

A STUDY OF COTTON GROWN UNDER CONSTANT SOIL MOISTURES

Part II. Dry Weight, Yield and Economy of Water

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1. Introduction

The effect of different moisture levels on the development of cotton plant has been discussed in Part I of this series. In general, it was found that Asiatic variety cannot tolerate very high moisture content in the soil, while the American variety is able to withstand soil moisture up to the field capacity. It was also shown that with the American variety transpiration increased with increased moisture in the soil while with the Asiatic variety the transpiration is maximum at 24% decreasing with further increase in the soil moisture. It was considered that it would be worthwhile to find out whether this differential response of the two varieties of cotton to the soil moisture is seen also in the total dry matter produced.

In this paper an attempt is made to study the effect of 3 levels of soil moisture, namely saturation (32%), three-fourth saturation (24%) and half saturation (16%) on (1) the growth and development of the American and Asiatic cottons with reference to the amount of dry matter and kapas; (2) the amount of water transpired to produce a unit quantity of dry matter as well as a unit quantity of seed cotton; and (3) the quality of kapas produced.

2. Material and Method

As already stated in Part I of this series, these experiments were carried out with a variety of Asiatic cotton (*Gossypium arboreum* L. var. *neglectum* forma *bengalensis*, an improved strain 43 evolved at Indore) and a variety of American cotton (*Gossypium hirsutum* L. M.U.$_4$). The technique followed to raise these plants in pots has been described in Part I of this study. The method used for the measurement of transpiration has also been described in the above paper.

As soon as the bolls were ready for picking the kapas were collected and stored in paper bags. The following determinations were made:

* The data discussed in this paper were collected by the author when he was a research worker at the Institute of Plant Industry, Indore, Central India, in the year 1944–45.

(i) yield from individual plants; (ii) ginning percentage. Yields of individual plants were ginned separately in small power driven gins specially designed to handle single plant yield; (iii) staple length. The staple length was determined by Bailey method; and (iv) fibre maturity. The maturity test was carried out as usual by counting hundred alkali treated fibres at random with the help of a microscope.

Throughout the growth period, any dry leaf, flower and flower parts and immature bolls which were shed, were collected daily for individual plants. When pickings were over the stock of the plant was severed at the cotyledonous nodes. This was then divided into the following parts:—(*a*) stalk without leaf lamina, (*b*) leaf lamina only, (*c*) reproductive parts of the plant. These three portions along with the plant parts, if any, shed and collected previously, were dried in a hot air oven at 98° C. till a constant weight was reached.

In the case of roots, soil was carefully washed away with a jet of water over suitable sieves to trap any tiny rootlets that may snap during the process of washing. The roots along with these rootlets were also dried in the oven at a temperature of 98° C. till constant weight was reached.

3. Dry Matter

Table I gives in grammes the mean dry matter per plant along with the critical differences of (1) whole plant, (2) root, (3) shoot, (4) stalk, (5) leaf lamina and (6) reproductive parts including buds, flowers and perianth, carpel and seed cotton.

Table I

Mean dry weights in gramme per plant along with the critical differences of the whole plant, root, shoot, stalk, leaf lamina and reproductive parts

Details (weight in gm.)	American (M.U.$_4$)				Asiatic (strain 43)			
	Level of moisture			C.D. at 5% level	Level of moisture			C.D. at 5% level
	16%	24%	32%		16%	24%	32%	
1. Whole plant ..	138·5	255·1	208·6	59·60	77·0	184·7	93·2	19·58
2. Dry weight of root ..	14·8	21·7	12·9	6·08	10·8	35·0	10·6	6·22
3. Dry weight of shoot	123·7	233·4	195·7	57·51	66·2	149·7	82·6	16·57
4. Dry weight of stalk..	29·0	53·0	43·1	12·50	13·9	47·5	22·2	5·66
5. Dry weight of leaf lamina	24·9	47·5	41·8	17·46	12·6	33·3	22·1	5·35
6. Dry weight of reproductive part	69·8	132·9	110·8	44·00	39·7	68·9	38·3	11·99

C.D. means critical difference.

The values given in Table I are shown in the form of a histogram in Fig. 1. It will be seen from Table I that for all the plant parts, the dry weight is maximum when the soil moisture was 24%. The differences in

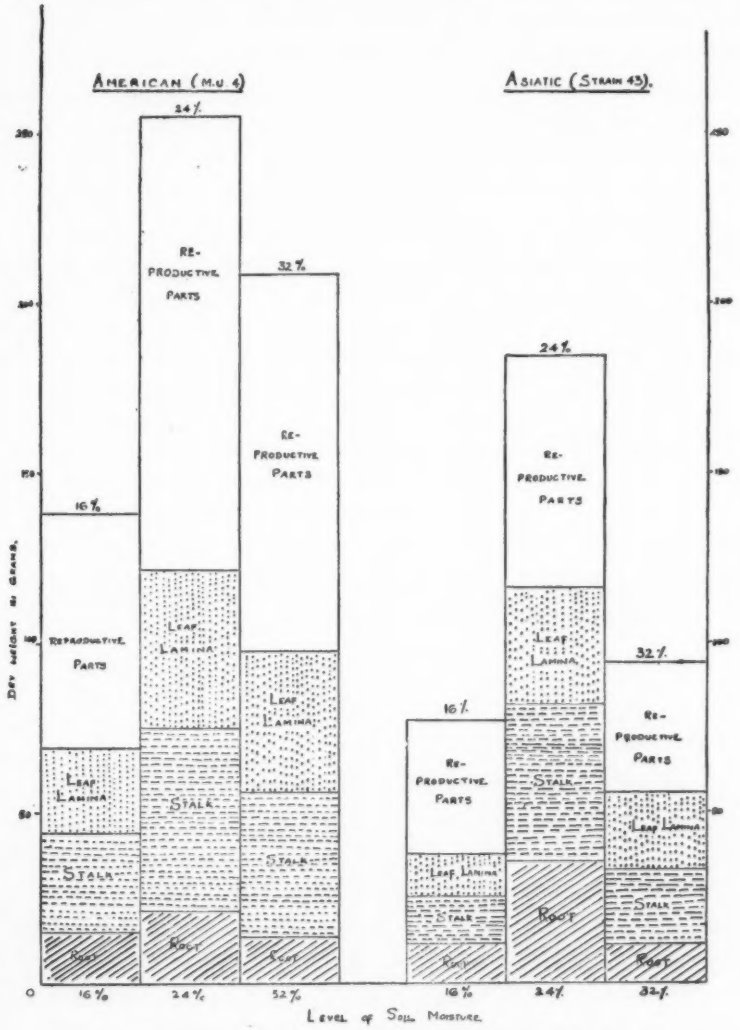

Fig. 1. Histogram showing the dry weight of various plant parts

the dry weights at 24% and 32% are, however, significant only in the case of the Asiatic but not so in the case of the American variety. This holds

good for all characters under observation except for the dry weight of the root. This seems to indicate that whereas the Asiatic variety markedly suffers with the increase of the moisture to 32% level, the American variety does not appear to be adversely affected.

4. Intensity of Transpiration

Table II gives (i) the total amount of water transpired by the plant during (1) complete life-cycle of the plants; (2) the period of elongation and leaf production only, along with the critical differences; and (ii) the ratio of the latter to the former.

TABLE II

Total amount of water transpired during (1) *the entire life cycle and* (2) *the elongation only in litres along with the critical differences and their ratios*

Details	American (M. U.$_4$)				Asiatic (strain 43)			
	Level of moisture			C. D. at 5% level	Level of moisture			C.D. at 5% level
	16%	24%	32%		16%	24%	32%	
1. Total amount of water transpired (in litres)	52·09	105·64	126·64	10·92	24·41	59·74	43·58	4·81
2. Water transpired during growth (in litres)	24·47	53·12	77·27	3·81	13·46	30·28	25·20	3·20
3. Ratio of second to first	·470	·503	·610	..	·551	·507	·578	..

C.D. means critical difference.

It has been pointed out in Part I of this study (1) that

(i) the grand period of elongation and leaf formation extended to eleven weeks in both the varieties but commenced from 8–9–1944 in American variety and from 15–9–1944 in the Asiatic variety; and

(ii) ratio of water transpired during the elongation and leaf formation period only to water transpired during its entire life-cycle.

It will be seen from Table II that (1) the American variety under study requires two to three times the amount of water transpired by Asiatic variety grown under corresponding levels of moisture.

(2) About 50% of the total quantity of water is transpired by both the varieties during the grand period of elongation and leaf formation.

(3) In both the varieties the transpiration loss at 24% is significantly more than that at 16%.

(4) The transpiration loss increases significantly with the increase of soil moisture from 16% to 24% and 24% to 32% in American, while in Asiatic there is a significant increase in loss from 16% to 24% and then a significant decrease from 24% to 32%. This may perhaps indicate that the capacity for the absorption of water by American variety is not affected adversely even when the soil moisture is 32% whereas in Asiatic variety it does so.

5. Transpiration in Relation to Dry Weight

In the present study the amount of water taken up by the plant has not been directly measured. But for all practical purposes it may perhaps be assumed that the transpiration loss represents approximately the water taken up. It was thought worthwhile to make an attempt to study the economy of water in the two varieties of cotton under the varying conditions of soil moisture. For this purpose the total quantity of water transpired during the complete life history of the plant was divided by the dry weight produced. Table III indicates the amount of water transpired in c.c. to build up a gramme of (1) root, (2) shoot, (3) stalk, (4) leaf lamina, (5) reproductive parts, (6) kapas and (7) entire plant.

Table III

Amount of water transpired in c.c. to build up a gramme weight of dry matter in the form of root, shoot, stalk, leaf lamina, reproductive parts, kapas and entire plant

Details	American (M.U.$_4$)			Asiatic (strain 43)		
	Level of moisture			Level of moisture		
	16%	24%	32%	16%	24%	32%
1. Root ..	3520	4866	9814	2259	1707	4111
2. Shoot ..	421	453	647	369	399	528
3. Stalk ..	1797	1992	2937	1755	1258	1963
4. Leaf lamina ..	2092	2223	3029	1937	1794	1972
5. Reproductive parts ..	746	795	1143	615	867	1135
6. Kapas (seed cotton) ..	1192	1187	1922	1191	1697	2519
7. Entire plant ..	372	414	607	317	323	466

The following inferences may be drawn from the above table:—

(1) The American variety requires 20% to 40% more water to produce unit dry matter of different plant attributes (except kapas) than the Asiatic

variety. In other words, the Asiatic plant shows a better economy of water in general.

(2) But to produce a unit weight of kapas, American plants require less amount of water.

(3) Considering plant as a whole, in both the varieties the ratio of transpiration to unit dry matter increased with the increase in soil moisture. This can be due to either an increase in the amount of water transpired or a decrease in the dry matter produced or both. It has been shown in Table I that in the American variety the dry matter produced at 32% is not significantly less than that at 24% soil moisture, whereas in the case of the Asiatic variety the dry matter at 32% is markedly less. Further from Table II it will be seen that the transpiration at 32% is lesser than that at 24% in the case of the Asiatic variety whereas this is more in the case of the American variety. It may thus be assured that the increase in the ratio of the total water transpired to total dry matter produced is due to total increased absorption (assuming transpiration to be an index of absorption) of the American variety in the presence of greater quantity of soil moisture, whereas in the case of the Asiatic variety this is due to smaller amount of dry matter produced.

In the investigation into the water requirement of crop plants Singh, *et al.* (1935) found that the water requirement, based on dry matter produced for Punjab American 4 F, to be 446 c.c. and for Jalgaon Banila No. 10 to be 399 c.c. These values agree quite well with the present findings of water requirement of cotton plants under 24% moisture level (the optimum soil moisture level). But the average dry matter produced per plant is rather very low.

6. Comparative Study of the Dry Matter produced by Different Plant Parts

It is evident from Tables I and III that the dry matter found in different plant parts is not in the same proportion in the plants grown under the three levels of moisture. It may be interesting to study not only the absolute values of dry matter found in different parts of the cotton plant but also the relative amounts of dry matter present in them. With this in view the ratios of dry matter present in different plant parts to (i) the total dry weight, (ii) the dry weight of shoot (total dry weight minus roots) are presented in Table IV. Also the ratios of the dry weight of kapas to the total dry weight of reproductive parts (flowers and fruits) are given in the last row.

TABLE IV

Ratios between different plant attributes

Details	American (M.U.$_4$)			Asiatic (strain 43)		
	Level of moisture			Level of moisture		
	16%	24%	32%	16%	24%	32%
1. Dry weight of root to total dry weight	·107	·085	·062	·140	·189	·114
2. Dry weight of shoot to total dry weight	·893	·915	·938	·860	·811	·886
3. Dry weight of stalk to total dry weight	·209	·208	·207	·181	·257	·238
4. Dry weight of leaf lamina to total dry weight	·180	·186	·200	·164	·180	·237
5. Dry weight of reproductive parts to total dry weight	·503	·521	·531	·516	·373	·411
6. Dry weight of kapas to total dry weight	·312	·349	·316	·266	·191	·186
7. Dry weight of root to shoot	·120	·093	·066	·163	·234	·128
8. Dry weight of stalk to shoot	·234	·227	·220	·210	·317	·269
9. Dry weight of leaf lamina to shoot ..	·201	·204	·214	·190	·222	·268
10. Dry weight of reproductive part to shoot	·565	·569	·566	·600	·460	·464
11. Dry weight of kapas to shoot ..	·278	·320	·296	·229	·155	·165
12. Dry weight of kapas to reproductive parts	·619	·670	·595	·516	·511	·452

These ratios bring out clearly the effect of different levels of moisture on the constituent parts of the plant.

The following inferences may be drawn:—

(1) The decrease in the ratio with increase in soil moisture definitely shows the depressing effect of higher soil moisture. Thus it may be noticed that ratio of root to total dry weight in American variety progressively falls while in Asiatic variety there is a large increase at 24% moisture level and then a sharp fall. It is but natural that with easy availability of soil moisture the plant is able to take in more water with less root development. Also the ratios for kapas in Asiatic variety fall with increase in soil moisture.

(2) The decrease in the ratio of some plant attributes naturally has to be reflected in increase in the ratio in certain other plant parts. So, we find slight increase in the ratios of lamina as well as the reproductive parts to dry weight in American variety. For Asiatic variety the ratio for leaf lamina alone increases with higher soil moisture.

In order to remove the influence of varying root development in different treatment on these ratios, the ratio of dry matter produced in different plant parts to total dry matter of shoot alone has also been worked out.

(3) After removing the contribution of root to total dry weight the ratio of reproductive parts to dry weight of shoot remains constant in American

variety and is of the order of 0·56 whereas in Asiatic variety the decrease in ratio from 16% to 24% soil moisture persists.

(4) The magnitude of the ratios within the same plant characters for the three levels of moisture are practically of the same order in American variety and this may perhaps substantiate the inference regarding the greater tolerance of the American variety for a wider range of soil moisture.

7. Yield

The following yield figures were collected: (*a*) Total weight of kapas (seed cotton), (*b*) weight of seed, (*c*) weight of lint and (*d*) total number of developed seeds per plant. Mean values per plant of the above plant attributes as well as the ginning percentage, mean weight of a seed cotton and mean weight of a seed along with their critical differences are presented in Table V.

Table V

Mean weight of kapas, lint and seed in grammes and ginning percentage along with their critical differences

Details	American (M.U.$_4$)				Asiatic (strain 43)			
	Level of moisture			C.D. at 5% level	Level of moisture			C.D. at 5% level
	16%	24%	32%		16%	24%	32%	
1. Total weight of kapas in gm.	48·6	96·4	72·4	34·08	23·3	40·2	19·5	10·73
2. Weight of seed in gm.	30·6	61·1	45·4	24·60	16·0	28·8	12·5	7·02
3. Weight of lint in gm	18·0	35·3	27·0	10·12	7·2	11·4	6·0	2·86
4. Ginning percentage ..	36·6	36·4	37·8	4·49	30·3	27·8	31·4	1·39
5. Total number of seeds	327	633	519	246·6	277	384	254	134·4
6. Mean weight of seed cotton in gm.	0·1491	0·1556	0·1398	0·01977	0·0845	0·1044	0·0735	0·02830
7. Mean weight of seed in gm.	0·0942	0·0976	0·0863	0·01296	0·0595	0·0753	0·0505	0·01985

C.D. means critical difference.

It may be seen from Table V that (1) the American variety grown under 24% soil moisture has yielded significantly more of (*a*) kapas, (*b*) seed by number as well as weight and (*c*) lint, than plants under 16% moisture, the order of merit being 24% to 32% to 16%;

(2) the Asiatic variety under 24% soil moisture has yielded significantly more of (*a*) kapas, (*b*) seed by weight and (*c*) lint, than those under 32% and 16% moisture, the order of merit being 24%–16%–32%. Also, the Asiatic variety under 24% moisture has produced significantly heavier seed and seed cotton than plants under 32% moisture.

(3) The conclusions drawn above are further evidence to the inference that the American variety under study is able to tolerate higher moisture levels in the soil.

(4) The significantly lower value of the ginning percentage in Asiatic variety under 24% soil moisture than those for plants under 16% and 32% moisture requires some explanation. It has already been pointed out in Part I of this series that the Asiatic plants under 16% and 32% soil moisture have produced rather undeveloped and lighter seeds and more immature fibres. (Also please refer to Table VI giving the maturity test.) Hence the ratio of lint to seed weight is more, leading to higher ginning percentage.

Table VI gives the mean halo length and maturity test.

TABLE VI

Mean halo length and mean percentage of fully mature, half mature, and immature fibres in cotton along with their critical differences

Details	American (M.U.$_4$)				Asiatic (strain 43)			
	Level of moisture			C. D. at 5% level	Level of moisture			C. D. at 5% level
	16%	24%	32%		16%	24%	32%	
1. Halo length ..	21·0	25·9	22·8	0·83	23·7	27·4	26·4	0·53
2. Fully immature ..	66·3	72·8	55·2	5·73	34·9	71·9	23·4	16·46
3. Half mature ..	10·0	11·3	9·9	1·35	7·2	8·6	7·3	1·02
4. Immature ..	23·7	15·9	34·9	5·37	57·8	19·4	69·3	16·70

C.D. means critical difference.

It may be concluded from the above table that (1) in both varieties plants under 24% moisture have produced significantly higher halo lengths than plants under other treatments and those under 32% moisture in its turn have given rise to significantly higher halo lengths than 16% moisture. This conclusion is very interesting and is of great importance to agronomist

(2) In both varieties percentage of mature fibres is significantly more and immature fibres is significantly less in 24% moisture than in other two levels, while percentage of immature fibres is significantly more in 32% moisture. Varieties grown under 32% moisture have a tendency to produce more of immature fibres.

8. CONCLUSION AND SUMMARY

1. For both varieties the dry weights of all plant parts under study are maximum in plants grown under 24% soil moisture (Table I).

2. The differences in dry weight at 24% and 32% are, however, significant only in the case of Asiatic variety (Table I).

3. About half the total quantity of water taken up during the entire life-cycle of both varieties is utilised during growth period (elongation and leaf formation) (Table II).

4. In both varieties the transpiration loss at 24% is significantly more than at 16%. Also the transpiration loss in American variety increased significantly with the increase in soil moisture from 24% to 32% while this loss decreased significantly in Asiatic (Table II).

5. The water requirement of American variety is 20 to 40% more than Asiatic variety, while American variety shows a better economy of water for producing a unit weight of kapas (Table III).

6. Twelve sets of ratios of dry weights between different plant parts to total dry weight or to dry weight of shoot were worked out. It was noticed that (*a*) this ratio for root falls with higher moisture level in American whereas in Asiatic there was a large increase in 24% over 16% level followed by a sharp fall in 32%; (*b*) the ratio of leaf lamina increases in both varieties; (*c*) the ratio of dry weight of kapas is greatest at 24% in American variety and 16% in Asiatic variety (Table IV).

7. In both varieties the 24% moisture has yielded the maximum weight of kapas, seed and lint (Table V).

8. Plants under 24% soil moisture have yielded significantly greater halo lengths in both varieties (Table VI).

9. In both varieties plants grown under 32% moisture level have produced significantly more of immature fibres. Maximum amount of mature fibres are produced at 24% moisture (Table VI).

10. Finally there is a strong evidence indicating that the American variety is not adversely affected with increase in soil moisture from 24% to 32% whereas the Asiatic variety markedly suffers with the increase.

9. Acknowledgements

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HOMOZYGOUS TRANSLOCATION OBTAINED IN THE SECOND GENERATION FROM MATERIAL TREATED WITH NEUTRONS

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In a previous publication we have (Kostoff, 1943–44) reported, that dry seed from *Crepis capillaris* treated with neutrons (20·000 r) gave similar cytological abnormalities like those treated with X-rays (20·000 r). More detailed studies showed that the abnormalities can be classified as follows: (1) various *chromosomal dislocations* of the type of translocations, deficiency, fragmentations and formation of chromosomes with two centromeres; (2) *chromosomal heteroploidy* of the type of monosomy, trisomy and polysomy, and finally (3) *polyploidy*. These were found in the cells of the embryonal tissue when the seed were germinated for two days. The same abnormalities were found when the seed were germinated at various intervals of several months after the treatment. The rate of growth of the germinated seed treated with neutrons was suppressed when compared with the control material (Figs. 1 and 2) but the seedlings grew much better than those of the seed treated with X-rays, probably due to the poorer penetration of the neutrons through the seed coat (*cf.* Kostoff, 1943–44). Three ecotypes were studied: (1) from Leningrad, (2) from Bucarest and (3) from Budapest. They reacted somewhat differently.

About 70% of the dividing cells had abnormal karyotypes at the second day of germination of the material treated with neutrons, while only 30% of the cells had normal karyotypes (Figs. 3, 4, 5, 6, 7 and 8).

It is easy to evaluate the effect of neutrons upon actively living tissues and the dividing plant, animal and human cells, considering the fact that the dosage for the dried seed is about 20 to 50 times larger depending on the seed and resp. on the actively living tissues and dividing cells.

The translocations from A-chromosome on C, and *vice versa*, from C on D and *vice versa* and from A on D and *vice versa* were often seen. Translocations from A-chromosome on its partner A-chromosome, from C on C,

and from D on D were also observed. There were cells, where single chromosomes were affected as well as such where all or almost all of the chromosomes (3 pairs = 6 chromosomes) were involved in the dislocation processes (and all transitional degrees). In one case the long arms of both A-chromosomes were shortened as far as the short ones (the heads). The chromatin material have been translocated at the distal end of a D-chromosome so that this appeared almost three times longer than the normal one. In the same cell one C-chromosome has been elongated (distal end) obviously on account of the second D-chromosome, so that only one C-chromosome was intact.

The non-reciprocal translocation from A-chromosome on the satellite of D represents a special interest. The normal D-Sat-chromosomes have at the long thinny end a very small chromomere. In this case a large piece of one A-chromosome, about as large as the head of C-chromosome, or even larger, has been non-reciprocally translocated, so that at the thinny end of one D-Sat-chromosome a large chromatin piece was hanging (Fig. 5).

When treated seeds growed further, many of the seedlings died. Very few plants remained alive from the material treated with X-rays. Somewhat more plants remained alive from the material treated with neutrons (Fig. 1, 2).

These plants had chimerical tissues and organs, concerning their chromosomal constitution. Heterozygous chromosomal translocations of various kinds were most frequently observed. Whole roots, branches and even plants were affected.

It was interesting for us to follow in the next generation one translocation from one D-chromosome on the short arm (head) on one A-chromosome (Fig. 8). The short end D chromosome, following translocation, was marked as *d*, while the elongated one (the elongated short arm so that the arms became almost equal) A-chromosome due to the translocation from D-chromosome, we have marked as Ad' (d + d' = D). The normal plants have a chromosomal formula AACCDD (Fig. 7). The heterozygous translocations—AAd'CCDd (Fig. 8). This plant formed ca. 28·1% abortive pollen (one flower had 376 normal and 154 abortive, another had 230 normal and 96 abortive, and a third one had 518 normal pollen and 189 abortive pollen, or in total 1,124 normal and 439 abortive pollen). The plant formed rarely one quadrivalent AAd'dD. The gametes formed should be most frequently (1) ACD, (2) ACd, (3) Ad'CD, and (4) Ad'Cd, the second being nonviable in the male gametes (abortive pollen).

Selfing ca. 20 flowers under bags, ca. 50 obviously normal seed were obtained. From the plants raised in the second generation, ten were studied cytologically. Only one of them was homozygous with new karyotype Ad'Ad'CCdd (Fig. 9). Four of the other were normal (AACCDD) and

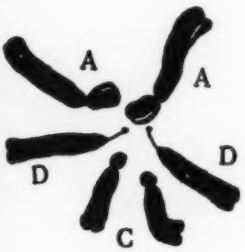

FIG. 7

FIG. 8

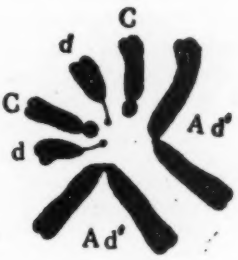

FIG. 9

four were heterozygous of the type AAd'CCDD. The last one was difficult exactly to determine, but most probably it seemed to belong to the heterozygous type AAd'CCDd (?)

Similar translocations of the type of AAd'CCDd occur occasionally in nature. The production of a homozygous plant with new karyotype indicates how evolution of the karyotypes may proceed in nature. Lewitzky (1931) maintained that the change of the chromosomes in nature usually goes from such with equal arms into such with unequal arm loosing gradually, chromatin. We have here one case, where the reverse process has occurred, namely the appearance of a homogygous new karyotype with one pair of chromosomes having almost equal arms, while the original plant had only chromosomes with heads.

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EXPLANATION OF FIGURES

FIG. 1. Upper row. Three tops with *Crepis capillaris* from Russia (Leningrad). The plants in the left top developed from untreated (control) seed, those in the middle—from seed treated with X-rays, those in the right top—from seed treated with neutrons.

FIG. 2. Middle row. Like Fig. 1, but the ecotype is from Hungaria (Budapest), left-untreated (control), in the middle X-rayed, right treated with neutrons.

FIGS. 3, 4, 5 and 6. Down. Photomicrographs of metaphases from the root tips of treated *Crepis capillaris* with neutrons. Figs. 3 and 4 are taken from one and the same cell

in unequal enlargements and at different levels, showing the translocation from chromosome *D* on the short arm of A. Fig. 5. The satellite of one D-chromosome is enlarged. Fig. 6. Translocation from one D (the short one) on one C, the latter being out of the focus.

FIG. 7. Normal karyotype of *Crepis capillaris* with 3 pairs of chromosomes = 6 chromosomes. Pair A has one long arm and a large head (small arm), pair C is shorter than A with smaller head and pair D is the Sat-chromosome pair, having a long arm, almost as long as that of chromosome A.

FIG. 8. Translocation from one D-chromosome on the short arm (head) of one A-chromosome (Ad'), so that a new karyotype appears with one normal A-ohromosome, one elongated A-chromosome = Ad', two normal C-chromosomes, one normal D-chromosome and one shortened D-chromosome = d.

FIG. 9. Homozygous translocation obtained in the subsequent generation from the heterozygous given in Fig. 8 with two new Ad' Ad' chromosomes (translocation from D on A), with two normal C-chromosomes and with two shortened chromosomes—d (due to translocation from D on A).

1
2

A SEPARATE CONTRACTILE MECHANISM FOR TONUS IN UNSTRIATED MUSCLE

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Unstriated muscle shows two kinds of tone, one which decreases and the other which increases in the absence of oxygen; the latter does not include the immediate increase of tone which may follow asphyxia and which is due to asphyxial hyperexcitability (Singh, 1949). In the present paper evidence is presented that these two properties of unstriated muscle are subserved by separate contractile mechanisms.

Experimental procedure was as described previously (Singh, 1949). Pieces of dog's stomach muscle were used.

Results

If the muscle is asphyxiated it may contract immediately; the tension then declines and may rise again. The former contraction is due to increase of lactic tone, and the latter to alactic tone (Singh and Singh, 1948 *b*; Singh, 1949). These two contractions may be differentiated by (1) change of temperature, (2) mechanically.

Effect of temperature.—If the temperature is lowered to 20° C., the first asphyxial contraction is increased and the second abolished (Fig. 1). Introduction of oxygen initially inhibits and then increases tone (Fig. 2). Glucose loses its inhibitory action, but oxygen produces this effect irrespective of the presence or absence of glucose, or if the muscle is poisoned with iodoacetic acid. At higher temperatures, the inhibitory action of oxygen decreases, but that of glucose is increased. The dog's stomach muscle at 20° C. resembles other muscles in which the second asphyxial contraction does not readily occur, such as fowl's gut, frog's stomach, guinea pig's uterus.

If the temperature of an asphyxiated muscle is increased, then the second asphyxial contraction occurs, but does not disappear on lowering of temperature again (Fig. 3). It disappears, however, if the temperature is raised to 50° C.

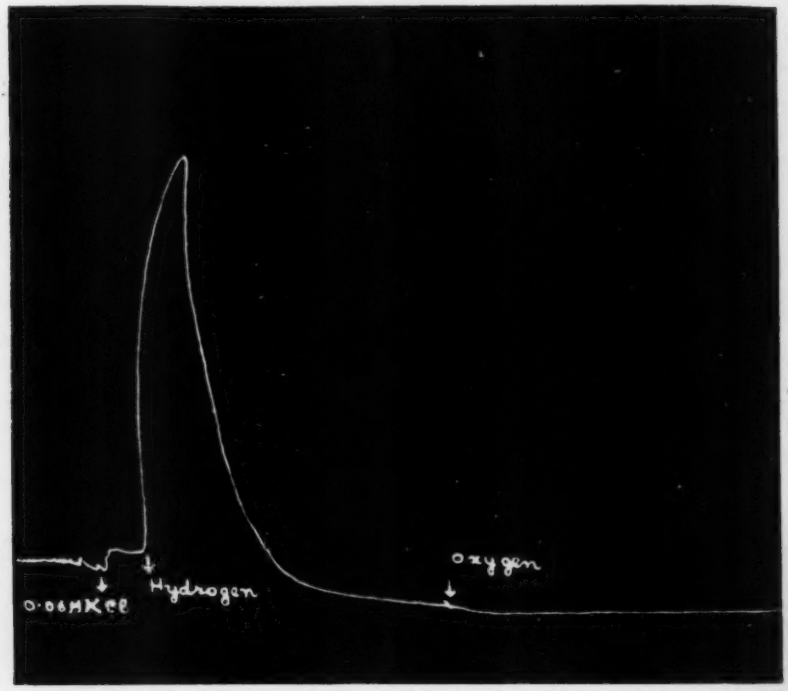

FIG. 1. Dog's stomach muscle, stimulated with potassium and then asphyxiated at 20° C.

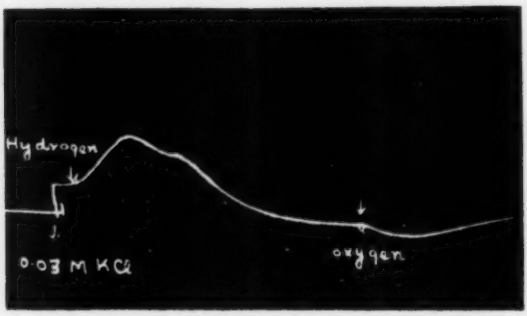

FIG. 2. Dog's stomach muscle, stimulated with potassium and then asphyxiated at 20° C. Drum stopped till potassium produces maximum tension.

At 20° C., neither cyanide nor iodoacetic acid produces a contraction, though cyanide may produce the first contraction. This shows that the contractions produced by asphyxia and iodoacetic acid are identical.

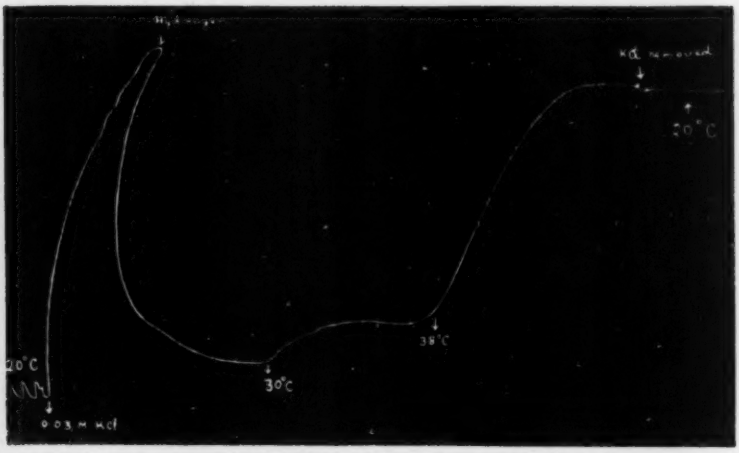

FIG. 3. Dog's stomach muscle stimulated with potassium at 20° C., and then asphyxiated. Temperature is then raised.

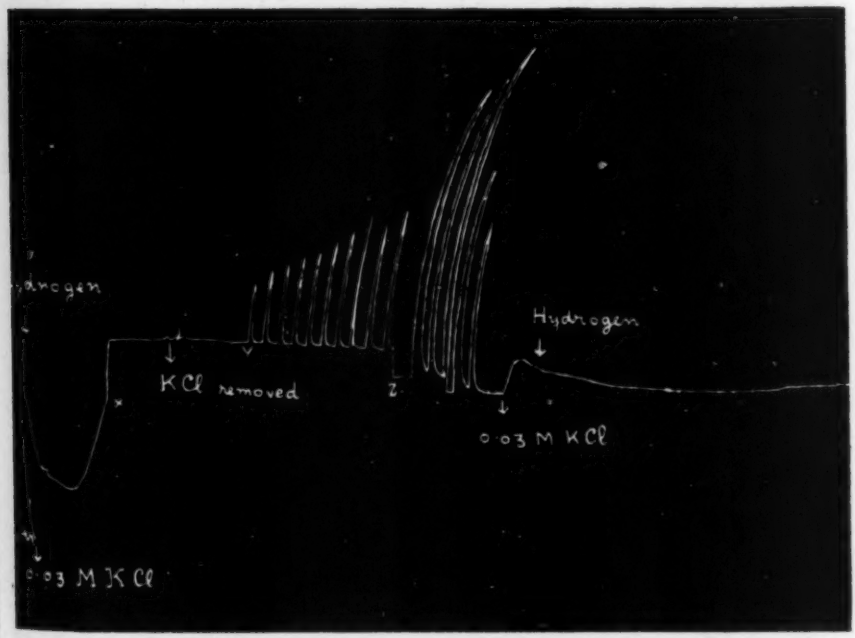

FIG. 4. Dog's stomach muscle. The asphyxial contraction slowly disappears. The muscle responds to alternating current. When stimulated with potassium again and asphyxiated the asphyxial contraction starts again. Drum stopped at x till asphyxial contraction develops maximally. Drum again stopped at z for 2 hours.

This second asphyxial contraction shows the following properties: (1) It is inhibited by oxygen (Singh and Singh, 1948 *a*); (2) It is inhibited

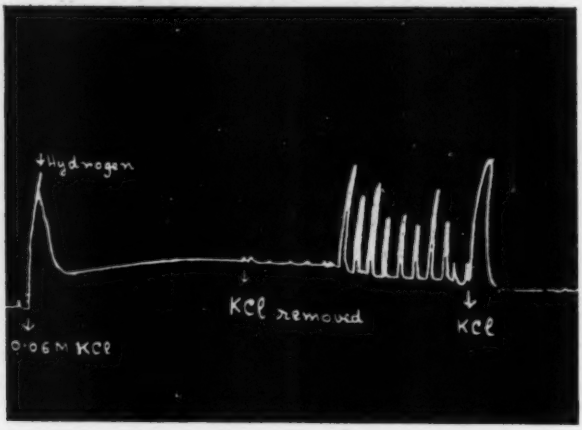

FIG. 5. Dog's stomach muscle. The effect of alternating current on the asphyxial contraction. After removal of KCl first time muscle stimulated with alternating current, 12 volts for 10 seconds per minute.

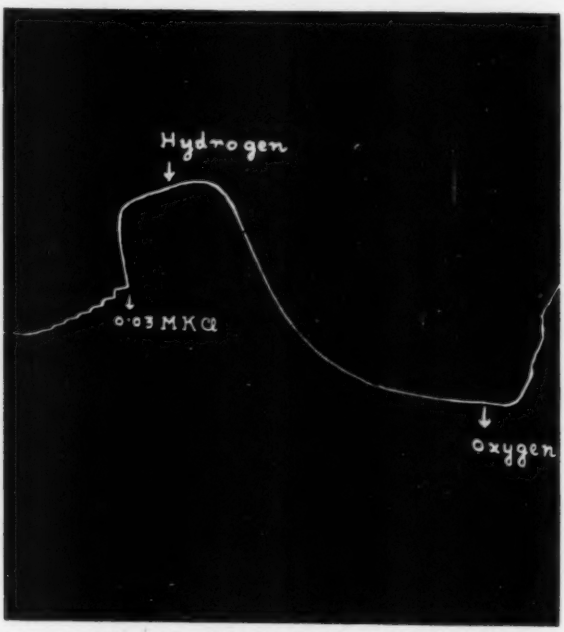

FIG. 6. Dog's stomach muscle. Stimulated with potassium and then asphyxiated. Previously it had been prevented from contracting during asphyxia.

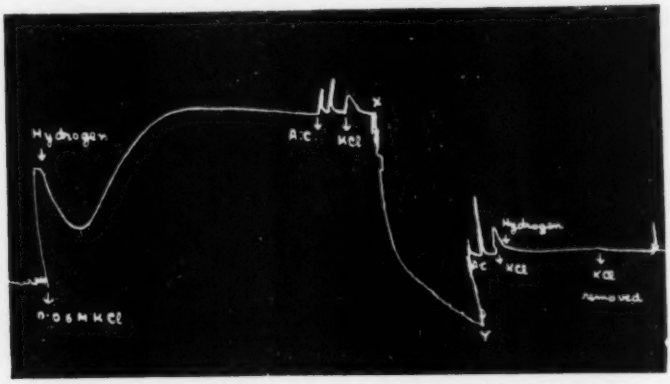

FIG. 7. Dog's stomach muscle. Isotonic lever. Asphyxiated, then forcibly stretched at x and released at y.

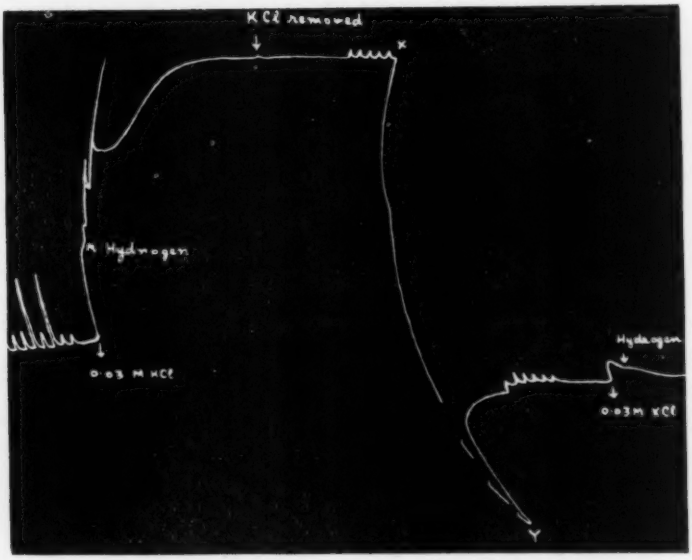

FIG. 8. Dog's stomach muscle. Isometric lever. Asphyxiated and then forcibly stretched at x and released at y.

by glucose (Singh and Singh, 1948 *b*; Singh, 1949). It may however pass into a state when it is unaffected by oxygen, glucose or asphyxia. Further contractions by alternating current, acetylcholine, potassium can be superposed on it, but inhibition by ammonium, potassium or adrenaline cannot be produced. This state is slowly reversible (Fig. 4). When not intense,

it may be partly abolished by alternating current (Fig. 5). This state could not occur normally owing to its slow reversibility.

Mechanical effects.—Using isotonic lever, the second asphyxial contraction can be prevented if the muscle is kept stretched and not allowed to shorten. The property of contraction on asphyxiation is permanently lost and the muscle behaves as it does at 20° C. (Fig. 6). While in asphyxial contraction, the muscle can be forcibly stretched (Fig. 7) or its tension destroyed (Fig. 8). The muscle then loses the power of asphyxial contraction, but retains the power of ordinary contraction by alternating current, potassium and acetylcholine. Potassium does not produce a sustained contraction.

Discussion

The effect of temperature and mechanical disruption shows the existence of a contractile system for tonic contraction, distinct from that for twitch contraction and lactic tone. In the muscle fibre there are two systems. These may be due to two proteins, or in the same protein there may be chemical groupings, which could cause it to contract in two ways (Singh and Singh, 1948 *b*). The muscle can thus contract to various degrees tonically, on which twitch contractions may be superposed.

The fact that skeletal muscle fibres are of two kinds, white and red, the latter being better adapted for tonic contraction than the former, and also the distinction between striated and unstriated muscles, leads to the conclusion that the same contractile mechanism cannot subserve both twitch and tonic contractions, hence the necessity for separate mechanisms. The gradation between white, skeletal, red skeletal and unstriated muscles suggests that the two contractile mechanisms are present in the same fibre, otherwise there would have been no necessity for such gradation, two kinds of fibres being sufficient for twitch and tonic contractions respectively. The same argument leads to the conclusion that these two functions are subserved by one protein, rather than by two proteins; the contractile protein becomes modified to perform tonic functions. From the various kinds of tonic contraction (Singh and Singh, 1946), it appears that there are several kinds of contractile proteins.

Summary

(1) At 20° C., dog's stomach muscle shows the first asphyxial contraction, but not the second.

(2) At 20° C., glucose loses its inhibitory action, but that of oxygen is increased; at higher temperature, the reverse happens.

(3) At 20° C., iodoacetic acid and cyanide also do not produce contraction.

(4) If the second asphyxial contraction is prevented mechanically or if developed, is abolished mechanically, then the power to contract on asphyxiation is permanently lost. Twitch contractions can be produced, but not tonic contractions. This suggests a separate contractile mechanism for tonic contraction (alactic tone).

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THE ACTION OF VARIOUS SUBSTANCES ON THE CONTRACTILE MECHANISM OF UNSTRIATED MUSCLE

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Most substances which produce contraction or excitation of unstriated muscle, do so by acting on the excitatory mechanism. A method has been described by which the reactions of dying muscles are studied (Singh, 1943). When a muscle dies it becomes permeable to all substances, so that they come into direct contact with the contractile mechanism. Under such circumstances it may react with these substances, and either contraction or active elongation is produced. The muscle, however, must be allowed to die naturally in saline; the contractile mechanism appears to be least disturbed thereby. The reactions of isolated myosin and intact muscles have also been compared (Singh, 1944 *a*). In the present research, the reactions of dying muscles have been studied, and thus the properties of the contractile mechanism investigated, being continuation of experiments described previously (Singh, 1944 *b*; Singh and Singh, 1946; 1949).

Experimental

Unloaded pieces of frog's stomach muscle were laid in a trough and left in various solutions for 24 to 48 hours unoxygenated, so that the elongation, if any, was active. Such muscles when restored to saline and stimulated were found to be inexcitable. Observations were also recorded after one hour's immersion, as these characterise the reactions of the living muscle which may be opposite those of the dying muscle. Pieces of frog's stomach muscle were cut out from the middle of the viscous and soaked in saline till they shortened to a constant length. This requires about half an hour. Each piece was then bisected longitudinally; one was used for the experimental solution and the other served as a control. The volume of solutions used for each piece was 100 c.c. The length was measured before and after soaking. With this procedure, the two pieces elongate or shorten almost exactly, the difference being not more than a mm. Pieces towards the cardiac or pyloric ends, tend to bend as they elongate, and thus shorten less and therefore the difference between the two pieces become greater. Therefore, pieces only from the middle of the stomach should be used.

Results

Effect of potassium.—Potassium causes the muscle to elongate by about 50 p.c., the effect of distilled water being to cause elongation by about 100–20 p.c. Distilled water is used as control, as it causes the greatest elongation of all substances tested. Thus in 6 muscles, the lengths due to active elongation in isotonic potassium chloride solution (0·112 *M* KCl) after immersion for 24 hours were 151 ± 20 p.c. of original, the corresponding lengths in distilled water being 211 ± 37 p.c.; the figures after immersion for one hour were 125 ± 6 and 154 ± 14 p.c. respectively. The muscle begins to elongate actively after the contraction is over. Immersion for one hour in potassium chloride does not cause permanent loss of excitability, which returns after restoration to saline. The fresh and dying muscles thus behave similarly. Small concentration of calcium counteract the effect of potassium. Thus in 6 muscles, the lengths in isotonic potassium chloride solution and in the presence of 0·0015 *M* $CaCl_2$ with the KCl, were 137 ± 10 and 123 ± 4 p.c. respectively after immersion for 24 hours; after immersion for one hour, they were only 106 ± 4 and 101 ± 4 p.c. respectively, calcium presumably diminishing the permeability to potassium.

The muscle fibres contain potassium as the predominant ion, so that it is presumably of importance in causing elongation. Concentration of potassium inside the fibres can be changed by varying the osmotic pressure of the saline (Gokhale and Singh, 1945). It is found that increase in osmotic pressure produces inhibition (Singh, 1939, 1942), whilst decrease has the opposite effect; in the latter instance, the water molecules may oppose the effect of potassium, and thus cause relaxation. In *Mytilus* muscle potassium causes marked relaxation (Singh, 1943).

Effect of ammonium.—Ammonium causes elongation by about 20 p.c., being less powerful than potassium. Thus in 6 muscles, the lengths after immersion for 24 hours in isotonic ammonium chloride solution (0·112 *M* NH_4Cl) and distilled water were 122 ± 13 and 236 ± 10 p.c. respectively; after immersion for one hour, they were 122 ± 8 and 161 ± 12 p.c. respectively. The muscle thus elongates maximally in ammonium chloride in about an hour. This is presumably due to greater permeability to ammonium than to potassium.

Effect of sodium.—Sodium chloride causes contraction. Thus in 6 muscles, the lengths after immersion for 24 hours in isotonic sodium chloride solution (0·112 *M* NaCl) and distilled water were 84 ± 14 and 220 ± 15 p.c. respectively. The muscle thus contracts in sodium chloride. Immersion for one hour produces opposite results, the lengths being 127 ± 8 and

156 ± 10 p.c. in sodium chloride and distilled water respectively. In sodium chloride, therefore, the muscle at first elongates actively and then contracts. The effect of sodium chloride is counteracted by small concentrations of calcium and potassium present in the saline. Thus in 6 muscles, the lengths after immersion for 24 hours in isotonic sodium chloride solution and in the presence of 0·0015 *M* $CaCl_2$ with the NaCl, were 79 ± 5 and 92 ± 9 p.c. respectively; after immersion for one hour, they were 129 ± 16 and 121 ± 7 p.c. respectively, calcium acting oppositely in the shorter period. Similarly, in 6 muscles, the lengths after immersion for 24 hours in isotonic sodium chloride solution and in the presence of 0·01 *M* KCl with the NaCl, were 77 ± 5 and 92 ± 9 p.c. respectively; after immersion for one hour, they were 129 ± 25 and 122 ± 7 p.c. respectively, potassium acting like calcium. The importance of the above finding with reference to tonus is apparent. Unstriated muscle contains more sodium than striated muscle and the former shows more tone than the latter (Singh, 1938 *a*).

Effect of lithium.—Lithium chloride produces less contraction than sodium chloride. Thus in 6 muscles, the lengths after immersion for 24 hours in isotonic lithium chloride solution (0·112 *M* LiCl) and distilled water were 97 ± 8 and 222 ± 39 p.c. respectively. As with sodium chloride, immersion for one hour produces opposite results, the lengths being 124 ± 9 and 153 ± 10 in lithium chloride and distilled water respectively. The power of elongation therefore by the monovalent ions varies in the order

$$Na < Li < NH_4 < K$$

Effect of hydrogen ions.—Elongation is slightly greater in alkaline than in acid saline, using phosphate buffer. Thus in 6 muscles, the lengths after immersion for 24 hours in saline of pH 5·2 and 8 were 92 ± 10 and 110 ± 13 p.c. respectively; immersion for one hour produced no significant difference. In distilled water there is an optimum at about pH 7, comparisons being made between solutions of pH 5·2, 6, 7, 8; there is no significant difference between the effect of pH 6 and 7. Thus in 6 muscles, the lengths after immersion for 24 hours in distilled water of pH 5·2 and 7 were 164 ± 16 and 201 ± 24 p.c. respectively; after immersion for one hour they were 127 ± 7 and 138 ± 6 p.c. respectively. In another 6 muscles, the lengths after immersion for 24 hours in distilled water of pH 7 and 8 were 203 ± 20 and 186 ± 16 p.c. respectively; immersion for one hour produced no significant difference, the lengths being 137 ± 9 and 137 ± 6 p.c. respectively.

Effect of divalent cations.—Calcium chloride causes slight contraction. Thus in 6 muscles, the lengths after immersion for 24 hours in isotonic

calcium chloride solution (0·07 *M* $CaCl_2$) and distilled water were 94 ± 7 and 209 ± 35 p.c. of original length respectively; immersion for one hour produced elongation in calcium chloride, the lengths in calcium chloride and distilled water being 110 ± 3 and 143 ± 12 p.c. respectively. Strontium chloride also causes contraction. Thus in 6 muscles, the lengths after immersion for 24 hours in isotonic strontium chloride solution (0·07 *M* $SrCl_2$) and distilled water were 61 ± 18 and 204 ± 24 p.c. of original length respectively; immersion for one hour produced elongation in strontium chloride, the lengths in strontium chloride and distilled water being 112 ± 4 and 150 ± 14 p.c. respectively. Barium chloride produces elongation. Thus in 6 muscles, the lengths after immersion for 24 hours in isotonic barium chloride solution (0·07 *M* $BaCl_2$) and distilled water were 106 ± 12 and 208 ± 29 p.c. of original length respectively; immersion for one hour produced no significant effect, the lengths in barium chloride and distilled water being 100 ± 6 and 144 ± 12 p.c. respectively. The effect of divalent cations in causing elongation, therefore, varies in the order

$$Ba > Ca > Sr.$$

Magnesium chloride causes elongation. Thus in 6 muscles the lengths after immersion for 24 hours in isotonic magnesium chloride solution (0·07 *M* $MgCl_2$) and distilled water were 126 ± 13 and 206 ± 23 p.c. of original length respectively; immersion for one hour produced the same elongation in magnesium chloride as in 24 hours, the lengths in magnesium chloride and distilled water being 126 ± 23 and 144 ± 10 p.c. respectively. In this respect the action of magnesium resembles that of ammonium.

Effect of anions.—Sodium bromide and nitrate caused slight contraction. Thus in 6 muscles, the lengths after immersion for 24 hours in isotonic sodium bromide solution (0·112 *M* NaBr) and distilled water were 95 ± 11 and 218 ± 23 p.c. respectively; after immersion for one hour, the lengths were 120 ± 10 and 152 ± 18 p.c. respectively. In 6 muscles the lengths after immersion for 24 hours in isotonic sodium nitrate solution (0·112 *M* $NaNO_3$) and distilled water were 94 ± 11 and 215 ± 44 p.c. respectively; after immersion for one hour, the lengths were 119 ± 15 and 149 ± 22 p.c. respectively. The effects of nitrate and bromide therefore, resemble those of chloride. Sodium iodide is indifferent. Thus in 6 muscles, the lengths after immersion for 24 hours in isotonic sodium iodide solution (0·112 *M* NaI) and distilled water were 99 ± 6 and 207 ± 15 p.c. respectively; after immersion for one hour, the lengths were 113 ± 4 and 145 ± 4 p.c. respectively. The effect of iodide thus resembles that of chloride. Sodium thiocyanate causes slight elongation, whilst sodium cyanide has a much

greater effect. Thus in 6 muscles, the lengths after immersion for 24 hours in isotonic sodium thiocyanate solution (0·112 *M* NaSCN) and distilled water were 119 ± 27 and 231 ± 33 p.c. respectively; after immersion for one hour, the lengths were 121 ± 9 and 166 ± 19 p.c. respectively. The effect of thiocyanate thus resembles that of ammonium and magnesium. In 6 muscles the lengths after immersion for 24 hours in isotonic solution of sodium cyanide (0·112 *M* NaCN) and distilled water were 240 ± 21 and 241 ± 15 p.c. respectively; after immersion for one hour, the lengths were 120 ± 15 and 160 ± 21 p.c. respectively. Sodium cyanide therefore causes as much elongation as distilled water. The effect of anions in causing elongation, therefore, varies in the following order.

$$Cl < Br = NO_3 < I < SCN < CN$$

Sodium bicarbonate causes marked elongation. Thus in 6 muscles, the lengths after immersion for 24 hours in isotonic solution of sodium bicarbonate (0·075 *M* $NaHCO_3$) and isotonic sodium chloride solution were 159 ± 20 and 74 ± 6 p.c. respectively; after immersion for one hour the lengths were 117 ± 14 and 132 ± 6 p.c. respectively. Bicarbonate is a normal constituent of the muscle, but the significance of this finding is not clear.

Sodium phosphate causes slight contraction. Thus in 6 muscles, the lengths after immersion for 24 hours in 0·056 *M* Na_2HPO_4 and isotonic sodium chloride were 89 ± 10 and 73 ± 2 p.c. respectively; after immersion for one hour the lengths were 87 ± 6 and 124 ± 7 p.c. respectively. In another 6 muscles, the lengths after immersion for 24 hours in 0·075 *M* Na_2HPO_4 and isotonic sodium chloride were 85 ± 7 and 65 ± 5 p.c. respectively; after immersion for one hour, the lengths were 64 ± 10 and 114 ± 9 p.c. respectively. In another 6 muscles, the lengths after immersion for 24 hours in 0·112 *M* Na_2HPO_4 and isotonic sodium chloride were 91 ± 14 and 70 ± 7 p.c. respectively; after immersion for one hour they were 68 ± 13 and 109 ± 9 p.c. respectively. In another 6 muscles, the lengths after immersion for 24 hours in 0·056 *M* NaH_2PO_4 and isotonic sodium chloride were 94 ± 10 and 83 ± 13 p.c. respectively; after immersion for one hour, the lengths were 123 ± 6 and 119 ± 10 p.c. respectively. In another 6 muscles, the lengths after immersion for 24 hours in 0·056 *M* Na_2HPO_4 and 0·056 *M* NaH_2PO_4 were 102 ± 13 and 97 ± 13 p.c. respectively; after immersion for one hour, the lengths were 65 ± 9 and 119 ± 8 p.c. respectively.

Phosphate is a normal constituent of the muscle fibres and has figured prominently in theories of muscular contraction. Phosphate thus causes

the muscle to contract, the alkaline more than the acid, the concentrations of phosphate within the range experimented upon, producing no significant difference. The immediate actions of the acid and alkaline phosphate are in opposite directions, the former causing active elongation and the latter contraction. The immediate effect of acid phosphate therefore resembles that of ammonium, magnesium and thiocyanate. These 4 substances are known to cause inhibition in unstriated muscle; inhibition therefore appears to be due to active elongation. Anions and some cations appear to produce a similar effect.

Sodium lactate has no significant action. Thus in 6 muscles, the lengths after immersion for 24 hours in saline without and with 0·1 p.c. sodium lactate were 117 ± 10 and 117 ± 8 p.c. respectively; after immersion for one hour, the lengths were 121 ± 11 and 125 ± 8 p.c. respectively.

Effect of drugs.—Acetylcholine causes contraction. Experiments were performed both in sodium as well as potassium salines. The latter medium was used, as potassium is the predominant intracellular ion. Acetylcholine thus appears to produce the same result by direct action on the contractile mechanism as through excitation. Thus in 6 muscles, the lengths after immersion for 24 hours in saline without and with acetylcholine (1 in 10,000), were 99 ± 10 and 84 ± 11 p.c. respectively; after immersion for one hour, the lengths were 100 ± 7 and 91 ± 6 p.c. respectively. In another 6 muscles, the lengths after immersion in potassium saline without and with acetylcholine (1 in 10,000) were 121 ± 10 and 99 ± 14 p.c. respectively; after immersion for one hour, the lengths were 92 ± 8 and 91 ± 7 p.c. respectively. This clearly shows that the action of acetylcholine is on the contractile mechanism, as excess of potassium renders the muscle inexcitable to acetylcholine; this is also shown by the one hour reading in potassium saline.

Pilocarpine produces contraction. Thus in 6 muscles, the lengths after immersion for 24 hours in saline without and with pilocarpine (1 in 10,000) were 108 ± 12 and 91 ± 14 p.c. respectively; after immersion for one hour, the lengths were 100 ± 11 and 108 ± 8 p.c. respectively. Nicotine and ephedrine have no significant effect. The action of adrenaline could not be tested, owing to its rapid decomposition.

Urea causes marked elongation, more than that produced by distilled water or any other substance. Thus in 3 muscles for each experiment, the elongation after immersion for 24 hours in solutions of urea of molar strength, 0·112, 0·224, 0·336, 0·672 was 217, 246, 254 and 255 p.c. respectively, the corresponding elongation in distilled water being 207, 228, 230

and 241 p.c. respectively. The elongation in one hour in urea was 147, 188, 161 and 161 p.c. respectively, the corresponding elongation for distilled water being 152, 202, 163 and 178 p.c. respectively. Urea is a normal constituent of body tissues, and its action is therefore interesting.

The action of normal alcohols is interesting; the action of methyl, ethyl, propyl, butyl, amyl and caprylic alcohol was tested. These alcohols counteract the effect of distilled water in causing elongation, though in small concentrations they have the opposite effect (Fig. 1). In this figure the effect

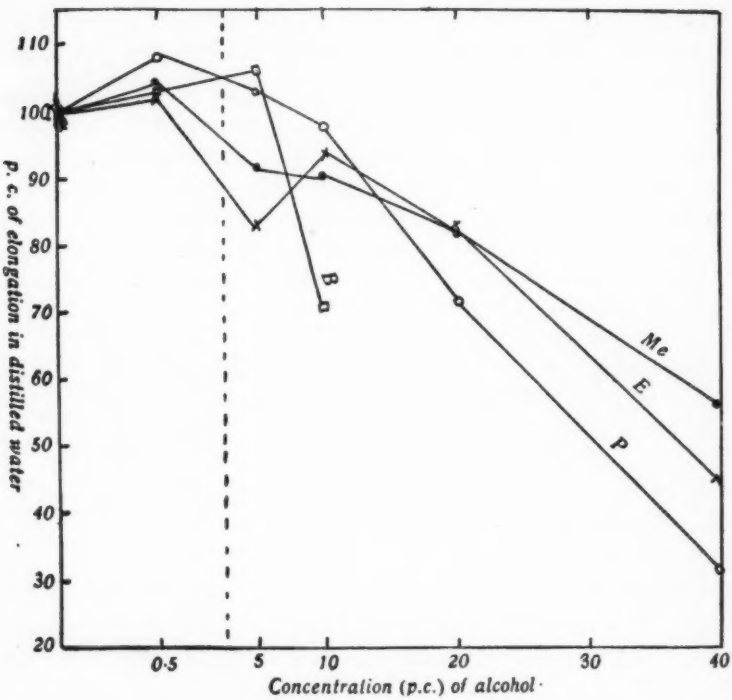

FIG. 1. Effect of alcohols on active elongation of frog's stomach muscle produced by distilled water.

Me = Methyl alcohol — *P* = *n*-Propyl alcohol
E = Ethyl alcohol — *B* = *n*-Butyl alcohol

of distilled water is represented as 100 p.c. elongation. Caprylic alcohol produced no significant action, probably owing to its limited solubility, and amyl alcohol only decreased elongation.

Discussion

One way of finding the action of substances on the contractile mechanism is to inject them into the muscle fibres; but excitation by this procedure cannot be excluded as the muscle membrane might be excited from within the fibres (Singh, 1938 *b*). Are the present findings of any significance? There is no doubt that the effect is on the contractile mechanism and some of these effects resemble those of ions within the fibres under normal conditions. In previous papers, the role of intracellular ions has been stressed, a subject which has not received as much attention as it deserves.

Effects by intracellular ions can be produced by varying their concentration by changes in the osmotic pressure of the saline. By such means, contraction, inhibition and effects similar to those brought about by electric current can be produced (Singh, 1942). Ions inside the muscle fibres produce effects similar to those produced by them outside the fibres.

The ions which are of significance are sodium, potassium, calcium bicarbonate, phosphate and acetylcholine as they are normal constituents of the muscle fibres. Unfortunately the effect of adrenaline could not be tested as it rapidly decomposes.

It would appear that combination of myosen with potassium causes elongation, and with sodium, contraction. Liberation of calcium would produce contraction. Thus in the muscle there are two substances, *viz.*, sodium and calcium which would cause contraction, and it is probable that they are responsible for tone and twitch contractions respectively. Whether acetylcholine is of extracellular or intracellular significance it is difficult to assess. The effect of phosphate lends support to the view that the combination of myosin with phosphate (from adenosine triphosphate) causes contraction.

These experiments also show that elongation during inhibition is active.

In *Mytilus* muscle, active elongation was not tested, but most substances that cause active elongation of frog muscle, cause relaxation in *Mytilus* muscle.

Summary

1. Potassium chloride causes active elongation of the frog's stomach muscle by about 50 p.c. by direct action on the contractile mechanism. This action of potassium is antagonised by small concentrations of calcium.

2. Ammonium chloride has a similar but less powerful action.

3. Sodium chloride causes contraction. This action is antagonised by small concentrations of potassium and calcium.

4. Lithium chloride produces slight contraction.

5. The power of the monovalent cations to cause active elongation varies in the order

$$Na < Li < NH_4 < K.$$

6. Active elongation is slightly greater in alkaline than in acid saline.

7. Active elongation in distilled water is maximum at about pH 7.

8. Calcium and strontium chlorides cause contraction.

9. Magnesium chloride produces active elongation.

10. Sodium bromide and nitrate cause contraction. Iodide is indifferent. Thiocyanate and cyanide cause active elongation. The power of the anions to cause active elongation varies in the order

$$Cl < Br = NO_3 < I < SCN < CN.$$

11. Sodium bicarbonate causes active elongation.

12. Sodium phosphate causes contraction.

13. Sodium lactate has no significant action.

14. Acetylcholine and pilocarpine cause contraction. Nicotine and ephedrine have no significant action.

15. Urea causes marked active elongation.

16. The effect of distilled water in causing active elongation is antagonised by methyl, ethyl, propyl, butyl and amyl alcohols. Caprylic alcohol has no significant action. Small contractions of methyl, ethyl, propyl and butyl alcohols have the opposite effect.

17. Active elongation occurs during inhibition produced by many substances.

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ON SOME INDIAN OCNERODRILIDS

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(Communicated by Professor Beni Charan Mahendra, D.Sc., F.Z.S., F.A.Sc.)

Introductory Note

Only a few Ocnerodrilids have been secured since closing a previous manuscript (Gates, 1945). Nevertheless those few enable extension of ranges of two species that are known only from India as well as recognition of one species of a new genus. A holandric species of *Malabaria*, now known from three localities in the United Provinces, still cannot be identified, or characterized specifically if new, because of lack of normal and clitellate material. These very interesting, rather annoyingly small species, may be sought in wet soils rich in organic matter.

Systematics

Family Ocnerodrilidæ

Genus *Deccania* gen. nov.

Diagnosis.—Male pores on xviii. Quadriprostatic, prostatic pores on xvii and xix. Quadrithecal, spermathecal pores on 7/8–8/9. Gizzards in vi–vii. Setæ closely paired.

Genotype.—Deccania alba sp. nov.

Distribution.—Central Provinces, India. Known at present only from the type locality of the type species.

Remarks.—Deccania is distinguished from *Malabaria* and *Thatonia*, the other Ocnerodrilid genera without extramural calciferous sacs, by presence of a second gizzard, in vi. Presence of dorsal pores and of a typhlosole are additional distinctions, so far as the single species now known is concerned. Persistence of male funnels in x indicates recent acquisition of the andric reduction which may or may not be characteristic of the genus.

Deccania alba sp. nov.

Material examined.—

Baraila (near Jubbulpore), Central Provinces, September, 3 late juvenile specimens. M. Matthur.

External characteristics.—Length, to 66 mm. Diameter, to 2½ mm. Number of segments, to 190. Unpigmented. On v–viii or ix a secondary furrow divides each segment into approximately equal halves with the setæ in the anterior half. From ix or x posteriorly additional furrows present. Prostomium apparently prolobous (retracted on each specimen). Setæ begin on ii and are closely paired; $ab = cd$, $bc < aa$, $dd\ ca = \frac{1}{2}$ C, ventral couples apparently lacking on xvii–xix (largest specimen). Dorsal pores present at least from 12/13 (3) but pore-like markings present in 11/12 or 10/11–11/12. Nephropores unrecognizable, possibly on or close to *b* lines.

Spermathecal pores unrecognizable but apparently on 7/8–8/9, on or close to *b* lines. Female pores on anterior margin of xiv just behind 13/14 and just lateral to *b* lines. Male and prostatic pores unrecognizable, the former may be on the setal arc of xviii somewhat median to *a* lines and the latter may be on setal arcs of xvii and xix in or near *ab*. No seminal grooves, or even rudiments thereof, recognizable. No demarcation of a male field recognizable.

Genital markings quite obvious, even on smallest specimen, but indistinctly demarcated, transversely placed, usually postsetal (and unless otherwise indicated), each with a quite obvious central pore. Each specimen has a pair of markings in region of *ab* on xx and on xxi (or possibly reaching across 21/22 on one worm). The smallest specimen has, in addition, a marking in *bc* on right side of ix, and another in the region of *ab* on left side of xiv. The second specimen has a marking in region of *ab* on right side of ix, another in region of *ab* on left side of xv but presetal, and a third apparently on 14/15 in region of *ab* on right side. The largest specimen has one extra marking, on x, in region of *ab* on left side.

Internal anatomy.—All septa from 5/6 posteriorly present; 5/6–8/9 thickly muscular, 9/10 muscular.

Gizzards in vi and vii (3). Gut in viii–xi moniliform, slightly widened and dark red with wall about equally thickened dorsally and ventrally in ix–x. Intestinal origin in xii (3). Typhlosole, which begins rather gradually in xiii or xiv, small but definitely lamelliform, becoming rather indefinite posteriorly and unrecognizable behind xc (1).

Circulatory system as in *Malabaria* and *Thatonia*, with last hearts in xi (3) and with no subneural trunk. Lateroparietal trunks unrecognizable.

Nephridia from xii or xiii large and reach nearly to mid-dorsal line. The long, slender duct passes into parietes behind septum about on *b* line. Nephridia of xi or xi and x small. Nephridia lacking or unrecognizable in x or ix anteriorly.

Metandric; testes and funnels free, testes large, male funnels flat discs on anterior face of 11/12 somewhat above parietes. A pair of smaller male funnels present in x (3). Seminal vesicles in xii (3). Prostatic duct *ca* one mm. long, narrower than gland, with slight muscular sheen. Vas deferens passes into parietes just in front of structures that appear to be follicles of ventral setæ of xviii and then continue mesially along setal arc for some distance before becoming unrecognizable.

Spermatheca with a rather irregular but fairly wide, saccular ampulla, and slender duct that is definitely longer than ampulla. A pair of small ovaries with few ova present in xii (1), but no female funnels present in that segment.

A gland of same appearance as prostates of xvii and xix is associated with each genital marking.

Regeneration.—The youngest specimen has at 57/58 a tail regenerate 7 mm. long, of 30+ segments, with a terminal anus.

Remarks.—Genital markings are unrecognizable on xvii and xix. In absence of such markings seminal grooves might be expected on more fully developed specimens were it not for the fact that in other Ocnerodrilid genera the grooves or furrows are often characteristically developed on late juveniles.

In absence of sexually mature material specific diagnosis can only be provisional.

Diagnosis.—Seminal grooves? Male field? Prostatic pores in *ab* (?) of xvii and xix. Male pores (on setal arc of xviii median to *a* lines?). Spermathecal pores on *b* lines. Genital markings postsetal, in *ab*, on xx, and xxi, additional markings on ix, x, xiv or xv. Setæ: $ab = cd$, $aa > bc$ $dd = \frac{1}{2}$ C, ventral setæ of xvii–xix lacking. Prostomium prolobous. Length 66 mm. Diameter, 2½ mm. Segments, 190.

Metandric but with persistent male funnels in x. Spermathecal duct slender and longer than ampulla. Genital marking glands like prostates.

Distribution.—Known at present only from the type locality in the Central Provinces.

Genus Malabaria Stephenson 1924

Malabaria sulcata Gates 1945

Material examined—

Robertsganj, Mirzapur district, United Provinces, September, 103 juvenile and 1 clitellate specimens. M. Matthur.

Rewa, Rewa State, Central India, October, 2 juvenile and 13 aclitellate specimens. M. Matthur.

Sohagi, Rewa State, Central India, November, 3 aclitellate specimens. M. Matthur.

External characteristics.—Of the 81 younger Robertsganj juveniles none have male porophores though the ventral setæ of xvii have been lost in each case. Two of these specimens do have recognizable rudiments of seminal grooves, longitudinal, about on *b* lines, extending across xvi–xvii. Male porophores on the clitellate specimen are normal, *i.e.*, diagonally placed and convergent posteriorly. Characterization of porophores of other specimens on which those markings are recognizable is shown below. Paired, longitudinal porophores are on xvii (2, Robertsganj), xvi–xvii (1, Robertsganj), left side of xvii–xix and right side of xvi–xvii (1, Rewa). Single longitudinal porophores are on xvii–xviii, reaching to the setal arc of xviii (2, Rewa) or 18/19 (1 Rewa and 1 Robertsganj).

MALE POROPHORES

	Number of specimens from		
	Robertsganj	Rewa	Sohagi
Both normal ..	14	9	..
One normal, left longitudinal ..	1	3	..
Both longitudinal ..	3	1	..
Both transversely placed, seminal grooves cresentic and concave posteriorly ..	4	..	3

Internal anatomy.—In each of the 25 dissections prostates and pyriform glands are again as in normal specimens, regardless of the shape and location of the male porophores.

Malabaria sp.

Material examined.—

Ahraura, Mirzapur District, United Provinces, October, 9 small and 1 large juvenile specimens. M. Matthur.

Remarks.—All specimens are holandric.

The large juvenile is aprostatic but several of the small juveniles have rudimentary prostates about as long as the nephridial ducts.

The large juvenile has a single spermatheca, opening on the right side about on the *a* line. The spermatheca is long enough to reach into xiii and a short ectal portion next the parietes is thickened and bulbous. In each of

three small juveniles there is on the right side a single rudimentary spermatheca, the ectal half thick and opaque, the ental half with thin transparent wall.

Genus *Thatonia* Gates 1942

Thatonia parva Gates 1945

Material examined.—

Allahabad, United Provinces, " drain in Daraganj quarter ", August, 10 juveniles. M. Matthur.

Rewa, Rewa State, Central India, October, 2 aclitellate specimens. M. Matthur.

Baraila (near Jubbulpore), Central Provinces, September, 1 clitellate specimen. M. Matthur.

External characteristics.—Length, to 45 mm. Diameter, to 2 mm. Behind the prostatic region *bc* is quite definitely smaller than *aa*, *dd* *ca*= or slightly $> \frac{1}{2}$ C. Clitellum on xiv–xxi.

None of the specimens have the supposedly characteristic genital marking on xx but sites of pores of genital marking glands are recognizable in *ab* on xx of the Baraila specimen.

Internal anatomy.—Genital marking glands of xx are present in four of the Allahabad specimens.

The dorsal blood vessel bifurcates in region of the brain, the branches uniting over the subpharyngeal ganglia to form the ventral trunk (several specimens).

Remarks.—One of the Allahabad worms is so young that prostatic rudiments are unrecognizable. Absence of genital marking glands of xx in five other specimens (in which rudimentary prostates are recognizable) may be real or only apparent due to glandular rudiments not yet having become visible in the cœlomic cavity.

Bifurcation of the dorsal blood vessel anteriorly and union of the branches ventrally to form the ventral trunk has been shown to be characteristic of several Magascolecid genera but has not been reported hitherto from the Ocnerodrilidæ.

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STUDIES IN CONTACT TOXICITY

Part III. The Effect of Pretreatment on Persistence of DDT on Cement Surfaces

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Received September 14, 1948

(Communicated by Dr. T. S. Subramanian, F.A.Sc.)

Introduction

Previous work carried out in these Laboratories[1] has indicated that Dichlorodiphenyl trichloroethane (DDT) and Benzene hexachloride (Gammexane) in oil-in-water emulsions can be mixed with a chalk-white wash to form effective insecticidal deposits on wall surfaces. The use of DDT and Gammexane in glue bound distempers[2] has also been reported. The application of DDT[3] both from emulsion and solution as a spray on cement surfaces has also been studied and a concentration of 150 mgm./sq. ft. has been found effective when tested against adults of *Tribolium castaneum* Herbst. At this stage, it was considered of interest to investigate the effect of pretreatment on the toxicity and persistence of the insecticidal deposits. Two substances, linseed oil and potassium dichromate/glue mixture, were initially selected for study. The present paper gives an account of the investigation with these materials. While the investigation was in progress, Parkin and Hewlett[4] and Hewlett and Parkin[5] have reported on the effect of pretreatment of (Fletton) brick with various materials like sodium silicate, gelatin, linseed oil, etc., on the toxicity of *Pyrethrum* in shell oil base. These workers have described the initial toxicity and persistence over a period of 23 days. The results embodied in the present paper relate to toxicity studies on DDT films on cement surfaces over a period of 13 months.

Materials and Treatments

1. *Cement Surfaces.*—Cement plates 6″ square and ½″ thick were used as the substrate for supporting pretreatments and insecticidal deposits.

2. *Pretreatments.*—These were as follows:—

(*a*) *Boiled Linseed Oil.*—The oil, thinned down with kerosene, Grade II, was applied uniformly on the surface with a camel-hair brush, at the rate of 787·5 mgm. per plate (3·15 gm. per sq. ft.). The plates were then dried for a week at room temperature and humidity.

(*b*) *Potassium dichromate/Glue mixture.*—The mixture had the following composition:

Hide glue	..	6	parts by weight.
Potassium dichromate	..	0·5	„
Water	..	93·5	„

(The function of the dichromate is to harden or insolubilise the glue.)

The mixture was uniformly applied with a camel-hair brush and the plates were dried for a week as in the case of linseed oil. The distribution of the dried material was 1·57 gm./sq. ft., and on each plate the concentration of glue and potassium dichromate were 363·7 mgm. and 30·3 mgm. respectively.

3. *Treatment with insecticide.*—Standard emulsion containing 5% (commercial) DDT[6] and a 10% solution of (commercial) DDT in kerosene, Grade II, were used. Mist deposition technique as described earlier [6] was considered to be the most suitable method of treatment in order to get uniform deposits. Initial sprayings were carried out to standardise the technique for the desired concentration of 150 mgm./sq. ft., and the sprays were absorbed on filter-papers of known area. The papers were kept raised from the bottom of the spray chamber by suitable supports, care being taken to ensure that the entire surface of the papers was normal to the direction of fall of the mist. The standardized conditions under which the concentration of 150 mgm./sq. ft. was deposited are given below:—

No.	Spray Liquid	Concentration used	Pressure used for spraying	Volume of liquid	Remarks
1	Emulsion	5%	20 lbs.	80 c.c.	Sprayed in two lots of 40 c.c. each giving 3 hrs. exposure to the mist each time
2	Solution	10%	20 lbs.	40 c.c.	Exposed for 4 hrs. to the mist

4. *Spraying.*—Three replicates in each of the plates, untreated, treated with linseed oil and treated with potassium dichromate/glue mixture, were used in each spraying operation. Four filter-papers were also exposed to the mist on each occasion and the concentration of DDT was assayed chemically with the following results:—

(i) Application from DDT kerosene solution 150·5 mgm./sq. ft.
(ii) „ emulsion „ 152·3 mgm./sq. ft.

In view of the difference in nature of the test substrate (cement plate) and the surface on which the insecticide was estimated (filter-paper) the above concentrations are only indicative of what must have been deposited on cement plates with and without treatments.

The sprayed plates were kept overnight for drying before entomological assay was carried out.

Entomological Assay

1. *Test Insects.*—Laboratory-bred adults of *Tribolium castaneum* Herbst. 4–5 weeks old, were used in the tests. The breeding of test insects was carried out in incubators at 34° C. and 70% R.H. In each breeding jar (glass beaker, 1 litre capacity, 8″ high and 4″ diam.) 500 adults were placed with one lb. of sterilized wheat flour. The grown-up 3rd and 4th stage larvæ were collected every week by sieving the flour through a 40-mesh sieve. The larvæ, so obtained, were kept separately, some 500 in each jar with wheat flour. (The adults were returned to the jars from which they had been removed.) The larvæ pupated in the jars and the adults emerged. The emerging adults were collected every week and kept on wheat flour till they were 4 weeks old, when they were used for tests. The stock breeding jars were examined once a month and dead insects replaced by fresh laboratory-bred adults. Wheat flour was also changed monthly in all the jars.

2. *Conditions of test and storage.*—The various tests and subsequent storage of plates were carried out in a partially conditioned room in which temperature and humidity ranged in the year between about 95 and 65° F. and 85 and 50% R.H. respectively.

3. *Exposure to insects.*—Batches of 20 insects were confined on plates by watch glasses, 5″ diameter, for 24 hours. After this period the insects were carefully removed and kept in small petri-dishes with a little wheat flour.

4. *Observations.*—Besides recording the mortality of insects immediately after the exposure period, daily count of mortality up to a period of 7 days was also made. Exposures on plates were carried out monthly for a total period of 13 months. At every exposure, control assay was carried out on a plate which had received no treatment whatsoever.

Results

The results are shown in Tables I and II and associated graphs Figs. 1 to 4. The mortality figures obtained at the end of 3 and 7 days above are presented in the tables. The figures represent the average of three replicates.

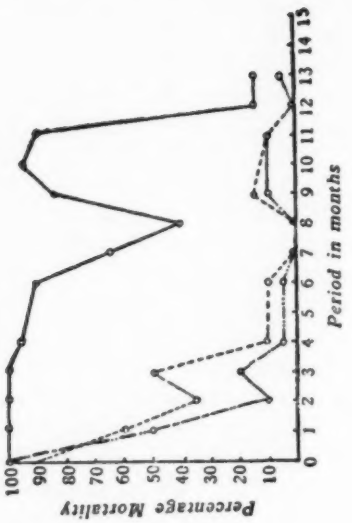
Table I. *Table showing percentage mortality on* Tribolium castaneum *Herbst. on cement surfaces treated with DDT in kerosene solution, with various pre-treatments*
(DDT concentration about 150 mgm./sq. ft.)

TABLE I. *Table showing percentage mortality on* Tribolium castaneum *Herbst. on cement surfaces treated with DDT in kerosene solution, with various pre-treatments*
(DDT concentration about 150 mgm./sq. ft.)

Pretreatment	Percentage mortality—months after treatment																										
	At the end of 3 days														At the end of 7 days												
	Initial	1	2	3	4	5	6	7	8	9	10	11	12	13	Initial	1	2	3	4	5	6	7	8	9	10	11	12	13
Linseed Oil	100	50	10	20	5	Not done	5	0	0	10	Not done	10	0	5	100	55	10	20	5	Not done	10	0	0	10	Not done	10	0	5
Potassium dichromate/ glue mixture	100	100	100	100	95	,,	90	65	40	85	95	90	20	20	100	100	100	100	95	,,	90	75	50	85	95	90	20	20
Nil ..	90	60	35	50	10	,,	10	0	0	15	Not done	10	0	0	90	65	35	50	10	,,	10	0	0	15	Not done	10	10	0
Control untreated	0	0	0	0	0	,,	0	0	0	0	0	0	0	0	0	0	0	0	0	,,	0	0	0	0	0	0	0	0

TABLE II. *Table showing percentage mortality* Tribolium castaneum *Herbst. on cement surfaces treated with DDT emulsion with various pretreatments*
(DDT concentration about 150 mgm./sq. ft.)

Pretreatment	Percentage mortality—months after treatment																										
	At the end of 3 days														At the end of 7 days												
	Initial	1	2	3	4	5	6	7	8	9	10	11	12	13	Initial	1	2	3	4	5	6	7	8	9	10	11	12	13
Linseed oil	100	95	40	35	15	Not done	5	5	5	15	Not done	15	5	5	100	95	40	35	15	Not done	5	5	5	15	Not done	15	5	5
Potassium dichromate/ glue mixture	100	100	100	100	95	,,	100	100	100	100	100	100	100	100	100	100	100	100	95	,,	100	100	100	100	100	100	100	100
Nil ..	100	85	30	35	15	,,	15	10	5	25	Not done	10	10	0	100	85	25	35	15	,,	15	0	5	15	Not done	10	10	0
Control untreated	0	0	0	0	0	,,	0	0	0	0	0	0	0	0	0	0	0	0	0	,,	0	0	0	0	0	0	0	0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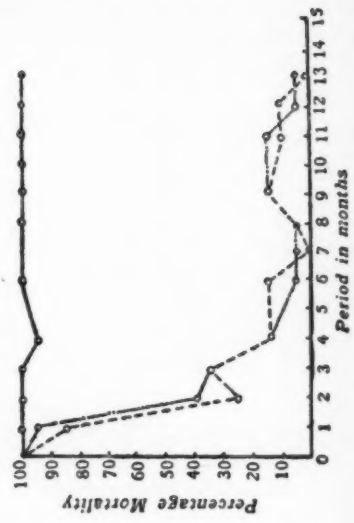
Period in months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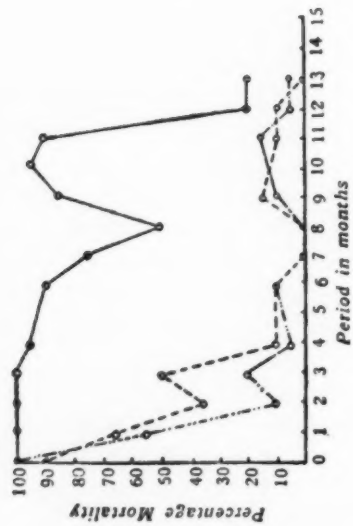
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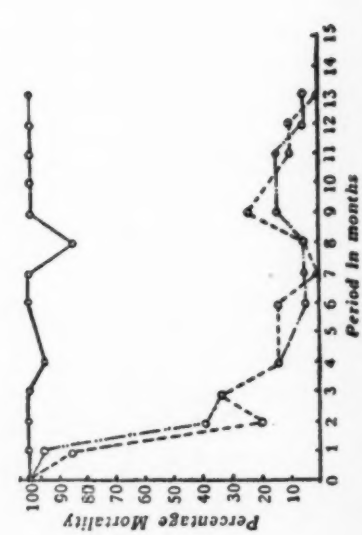
Period in months


Percentage Mortality
100 90 80 70 60 50 40 30 20 10
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Period in months

Discussion

In the beginning all treatments give uniformly high kills, but a striking difference is manifest in later exposures. Even from the first month onwards a regular decline in potency of surfaces pretreated with linseed oil and those not pretreated is observed. In the second month the mortality figures generally fall below 50% and in later months a very fast decline is noted. Further in the case of plates treated with DDT in kerosene solution, the effect of pretreatment with (boiled) linseed oil would appear to decrease the efficiency of the insecticide particularly in the first few months. As compared to these, the plates pretreated with potassium dichromate/glue mixture, generally maintain a high kill. This effect is observed in the case of deposits from both DDT emulsion and solution up to 11 months, after which the deposits from DDT solution lead only to a mortality of 20%, whereas those from DDT emulsion continue to give high kill.* In so far as a comparison is possible between these results and those of Hewlett and Parkin,[5] they are similar in nature, in that the findings of these workers with respect to gelatin and linseed oil pretreatments are supported by the present work.

Quite apart from the effect of pretreatments, in all cases an effective mortality is obtained in 3 days after exposure. There is only a slight increase in the intervening period upto 7 days.

The practical importance of the results will be readily appreciated. Where possible, pretreatment of cement surfaces with dichromate/glue prior to application of DDT is to be recommended.

Conclusion

Pretreatment of cement plates with potassium dichromate/glue mixture increases the persistence of DDT. On the other hand, pretreatment with linseed oil appears to have a slight reverse effect.

Acknowledgement

The authors' thanks are due to Dr. T. S. Subramanian for his interest in the work and to the Deputy Chief of General Staff (Weapons and Equipment) and the Director of Technical Development, Army Headquarters, New Delhi, for kind permission to publish the paper.

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* *Note added in proof* (3-10-49).—Results to date show that deposits from DDT emulsion continue to be active (26 months) giving 100% kills.

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FOLIAR SCLEREIDS IN SOME SPECIES OF *MEMECYLON* L.

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Introduction

Foliar sclereids or spicular cells have been reported to occur in several families among the Dicotyledons as is indicated by the accounts given by Solereder (1908). Earlier workers have recorded the foliar sclereids, but they failed to discern the importance of variations of the sclereids often occurring in one and the same species. The possible value of the foliar sclereids in the diagnosis of species was hinted upon by Solereder (1908). Much interest has been evinced on the study of foliar sclereids by the recent investigations of Foster (1946–47), who has contributed considerably towards the knowledge of our subject. He demonstrated the presence of the sclereids in the terminal position of the veinlets in several species of *Mouriria* Aubl. and *Memecylon* L. He states that the relation of the various sclereid-types to the veinlets in *Memecylon* needs a comparative study because of the probable close systematic relationship of this genus to *Mouriria*. This relationship was already stressed by Van Tiegham as far back as 1891 who placed the two genera under the sub-tribe " Mouririales " under the family " Melastomaceæ ". The present work was undertaken with a view to study the nature of the foliar sclereids in some of the available Indian species of *Memecylon*. Studies on ten species of *Memecylon* collected in South India are presented here.

Materials

The materials for this paper are mainly from the leaves collected and also from dried specimens deposited in several herbaria. The following are the plants examined:—

1. *Memecylon capitellatum* L. From the Herbarium, Government Botanic Gardens, Bangalore.
2. *M. depressum* Benth. .. Korekottae, Thirthahally, Mysore State Forests.

3.	*M. edule* Roxb. ..	Bannerghatta, Bangalore.
4.	*M. grande* Retz. ..	Kemmangundi, Mysore State Forests.
5.	*M. Heyneanum* L. ..	Government Botanic Gardens, Bangalore.
6.	*M. Lushingtoni* Gamble ..	Madras Herbarium, Coimbatore.
7.	*M. malabaricum* Cogn. ..	" "
8.	*M. terminale* Dalz. ..	" "
9.	*M. umbellatum* Burm. f. ..	" "
10.	M. species ..	Kemmangundi, Mysore State Forests.

Methods

Dried leaf-specimens were cleared as suggested by Foster (1946). Before dehydrating, it was found desirable to dip the material in acid-alcohol. Then the leaves were dehydrated, cleared in xylol and mounted in canada balsam without staining. For studying the shapes of the sclereids the cleared leaves were macerated and the sclereids thus isolated were mounted directly in a dilute solution of cotton blue in lactophenol. The mounts were made permanent by the modified method suggested by Thirumalachar (unpublished data).

General Distribution of Sclereids in the Lamina

The study of the cleared leaves, hand sections and macerations reveals that the sclereids are abundantly present in all the species so far investigated. The noteworthy feature in their disposition within the mesophyll is their close association with ultimate ends of the veinlets (Pl. XXVII, C, D and E). A careful examination reveals that within one and the same species the sclereid may be terminal, sub-terminal or diffuse in their distribution.

Sclereid Types

In the present study two main types of sclereids have been observed. In eight of the ten species of *Memecylon* investigated in the present study, the sclereids are ophiuroid, presenting an elongated columnar type of structure. In contrast, the other two species show polymorphic type of sclereid exhibiting a wide range of variation in " cell form ". The characters of the two types designated above are the following.

Type I.—The sclereids in this type are all ophiuroid and are relatively of the same shape. The cells are elongated with fusoid ends, sinuous or recurved in shape, with a narrow lumen of uniform width. The cell wall is homogeneous without manifesting lamellose structure or pits. Illustrations of this type are the following species: *Memecylon capitellatum*,

M. depressum, M. edule, M. grande, M. Heyneanum, M. malabaricum, M. terminale and *M. umbellatum.* Such a feature has also been reported by Foster (1946) in some American species of *Memecylon.* In addition to their terminal position, some of them are sub-terminal or diffuse. Sometimes more than one terminal sclereid run parallel to the veinlets for some distance closely adpressing to it. The diffuse sclereids show a tendency to incurve towards the veinlet, being closely adpressed to it to some distance.

In the subepidermal region of the leaf strands of sclereids which intertwined and run parallel to each other have been noticed (Pl. XXVII, A). They are often found to be situated in close association with the midrib. In other parts of the lamina, the sclereids are distributed in a criss-cross manner (Pl. XXVII, B and F). In a trans-section of the lamina of *M. Heyneanum* the sclereids traverse the mesophyll in all possible ways often running between the epidermis and mesophyll cells (Fig. 1). Such a feature is common in the other species of *Memecylon* having ophiuroid cell forms. Rao (1947) records such a condition of sclereid disposition in some species of *Olea.* The presence of fusoid endings near the stomata is an interesting feature conspicuously seen in the cleared mounts, and their significance is as yet unknown.

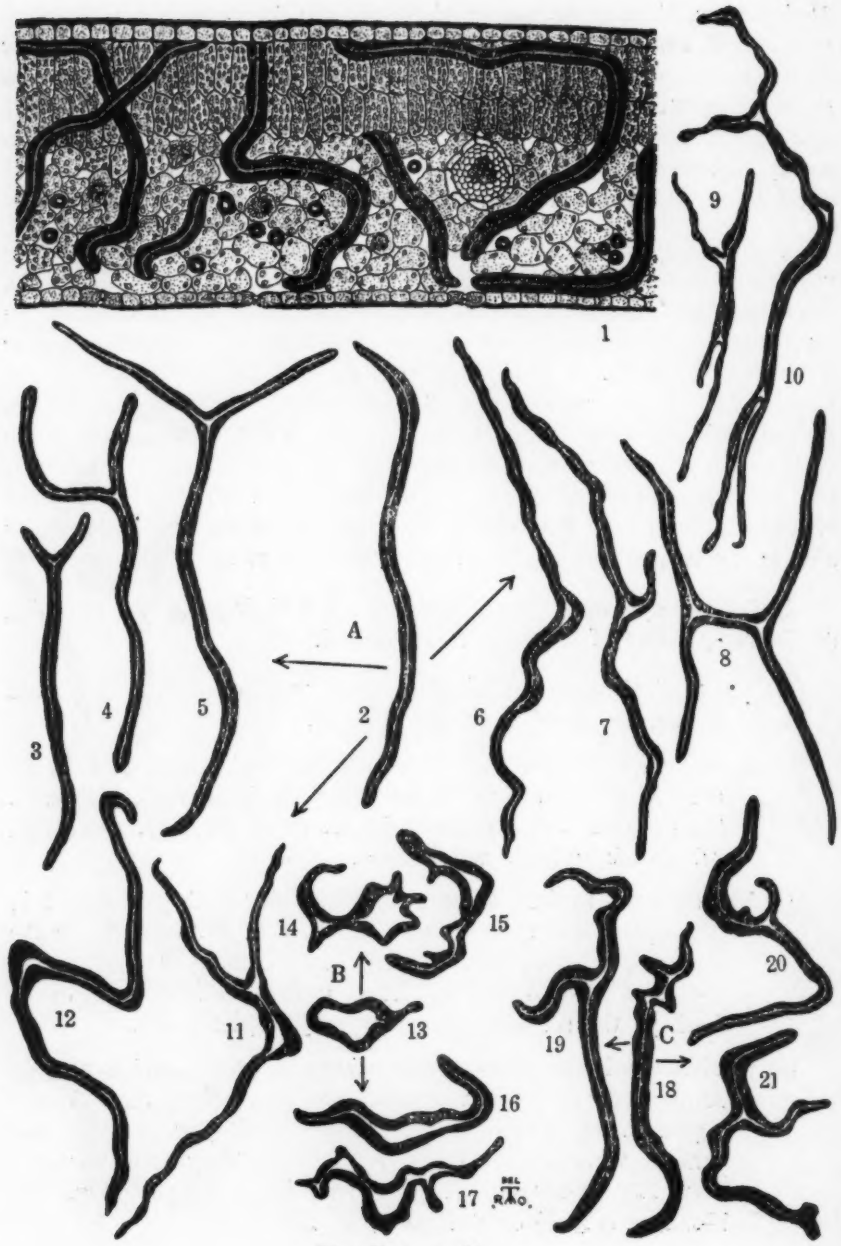
Under the ophiuroid type, depending upon their mode of branching the following forms of sclereids are recognised.

(1) Unbranched columnar elongated ophiuroid sclereids with fusoid endings as in *M. terminale* (Fig. 2).

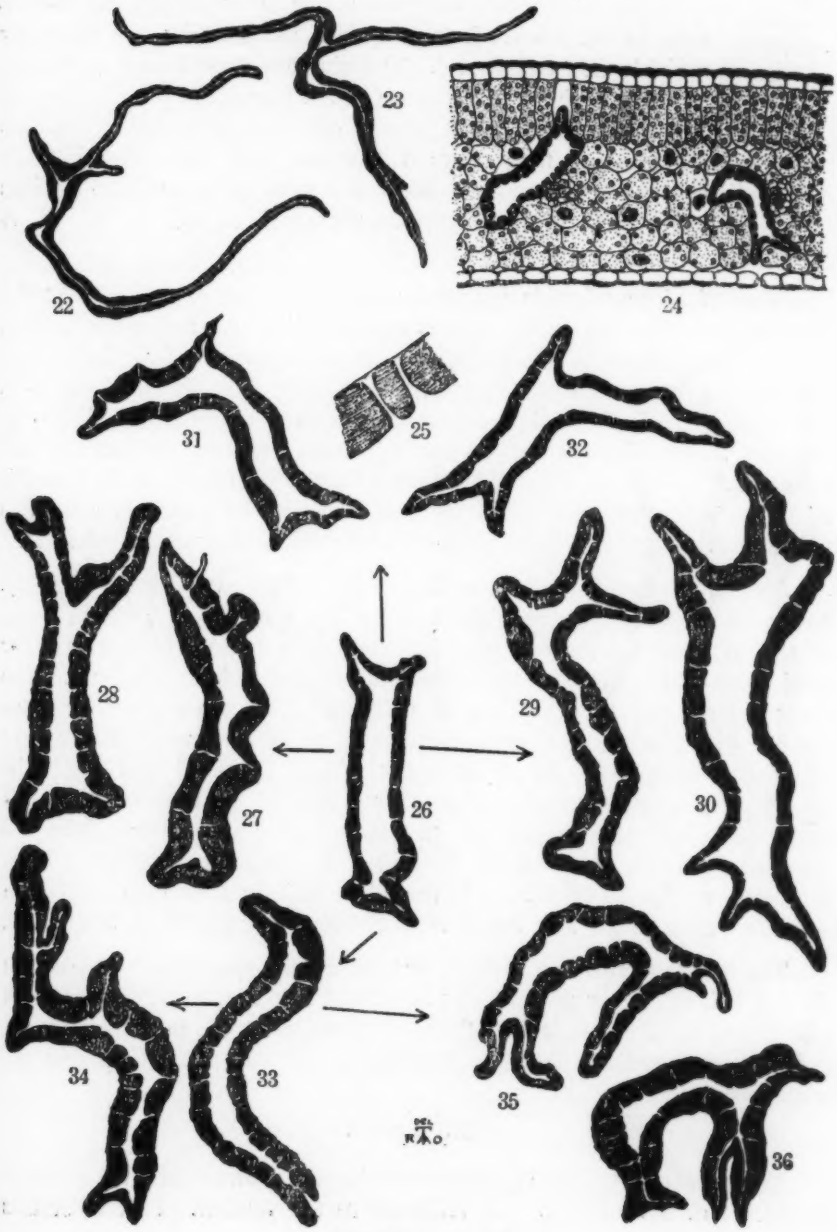
(2) Same form as (1) but there is a tendency to dichotomise at the tip (Fig. 5) or possessing short branches with fusoid endings (Fig. 3). Such sclereid forms are seen in *M. Heyneanum, M. depressum, M. grande* and *M. malabaricum.*

(3) The tendency to fork as seen in (2) is very well pronounced in *M. capitellatum* and *M. umbellatum.* In *M. capitellatum* the branched sclereids exhibit pronounced dichotomy resulting in Y-shaped sclereids (Fig. 4). In *M. umbellatum* in addition to the Y-shape the sclereids also show polymorphic forms.

(4) Ophiuroid sclereids showing great range in 'cell form' is seen in *M. umbellatum.* When isolated by maceration, the sclereids appear in the form of fusoid ophiuroid cells (Fig. 6) which are bent in various ways (Figs. 7 and 22). They are cruciform (Fig. 22), X-shaped (Figs. 9 and 10), Y-shaped (Fig. 11), H-shaped (Fig. 8) or T-shaped (Fig. 23).



TEXT-FIGS. 1—21



TEXT-FIGS. 22—36

(5) Finally the polymorphic existence of the ophiuroid sclereid form is seen in *M. edule*. In this species the sclereids are extremely variable in 'cell form'. The simplest type is the spheroidal cell with a short fusoid arm (Fig. 13), and the complex type is the ophiuroid cell bent in curious ways (Fig. 18), sometimes even forking to produce a Y-shaped cell form (Fig. 21). In between these two extreme cell forms, there are also various intergrading forms of curious shapes (Figs. 14, 15, 16, 17, 19 and 20).

Type II.—The sclereids included under this type are of interest for their polymorphic cell form. This is exemplified in *M. lushingtonii* and in a species of *Memecylon*. In a trans-section of the lamina of *M. lushingtonii* the sclereids are idioblastic in their disposition (Fig. 24). In *M. lushingtonii* the sclerosed wall of the mature sclereids exhibit a clearly stratified appearance (Fig. 25). The cell wall possesses numerous slender pit-canals, which are curved and oblique (Fig. 29). Such a feature is noticed in the sclereids of *Camellia* (Foster, 1944). All the variations can be traced back to two well-defined forms, *viz.*, osteo-sclereids and ophiuroid or astero-sclereids.

The osteo-sclereids exhibit two distinct lines of cell modification (Fig. 26). Along one line there is pronounced dichotomy resulting in Y-shape (Figs. 27 and 28), which undergoes bending (Fig. 29) and develops more slender fusoid branches resulting in the formation of a lobed body (Fig. 30). The latter is large in size with sclerosed walls and a large lumen of irregular width. Along the other, there is pronounced growth of one of the arms at opposite ends, resulting in an irregular shape (Figs. 31 and 32). The ophiuroid or astero-sclereids are short and bent in the form of a S-shape (Fig. 33). Here again, there are two main variations. In one form the S-shaped ophiuroid cell forks at the ends and forms fusoid branches from the middle of the cell (Fig. 34). In the other, such fusiform cells display an irregular curved shape with fusoid arms of unequal length (Figs. 35 and 36).

The brachysclereids are seen in an unidentified species of *Memecylon* (Pl. XXVII, D). They are of short form with fusoid ends. They exhibit a tendency to fork, but it is not of the pronounced type. In shape they are T-shaped or U-shaped with a non-stratified cell wall, and possessing pit canals.

Conclusions

The present study in ten species of *Memecylon* shows clearly the terminal disposition of the sclereids with reference to the veinlets. Further studies

are necessary to throw light on the exact significance of this interesting feature.

According to Stevens (1924) " the sclereids give mechanical toughness in the leaves against shearing stresses, without being an impediment to their increase in size ". Rao (1949) confirmed their function as structures providing mechanical toughness to some extent. The disposition of more than one sclereid in juxtaposition with the veinlets, showing reticulate arrangement in the mesophyll noticed in the present study, indicates their mechanical nature, thus affording additional rigidity in the leaf-blade.

Since the sclereids are abundantly present in *Memecylon* a detailed survey of the sclereids on the remaining Indian species, along with additional features like floral structures and histology of the leaf and stem as suggested by Foster (1946), will greatly help in building up an artificial key for the identification of the various species of this genus.

In conclusion, we wish to thank Prof. L. N. Rao and Prof. F. M. Bharucha for kind encouragement. Our thanks are also due to Dr. M. J. Thirumalachar for kindly going through the manuscript and for offering us valuable suggestions. We also thank Sri. S. N. Chandrasekharan, Systematic Botanist, Coimbatore, and the Curator, Government Botanic Gardens, Bangalore, for lending us herbarium leaf-specimens.

Summary

The mature sclereids occurring in ten Indian species of *Memecylon* are presented in this paper. They are terminal in position in relation to the ends of the veinlets. The different types of sclereids are described in detail. They serve a mechanical function to some extent. Their probable importance in building up a key for species of *Memecylon* on the basis of vegetative structures is pointed out.

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Explanation to Figures

Figs. 1-21. Fig. 1. Trans-section of the lamina of *Memecylon Heyneanum* showing the disposition of the ophiuroid sclereids. × 150. Fig. 2. Unbranched ophiuroid sclereid of *M. terminale*. × 120. Fig. 3. Sclereid of *M. Heyneanum*. × 129. Fig. 4. Sclereid of *M. capitellatum*. × 120. Fig. 5. Sclereid of *M. depressum*. × 120. Figs. 6–12. Polymorphic sclereids of *M. umbellatum*. All × 120 each except Fig. 12 × 129. Figs. 13–21. Polymorphic sclerids of *M. edule*. All × 129 each.

(*Note*.—The arrows indicate the probable lines of sclereid modification from the simple types designated here A, B and C.)

Figs. 22–36. Figs. 22–23. Sclereids of *M. umbellatum*. × 120 each. Fig. 24. Trans-section of the lamina of *M. lushingtonii* showing the idoblastic sclereids. × 200. Fig. 25. A portion of the sclereid wall to show the stratified nature and pit canals. × 1455. Figs. 26–36. Polymorphic sclereids of *M. lushingtonii*. × 301 each.

(*Note*.—The arrows indicate the probable lines of sclereid modification, from the simple type.)

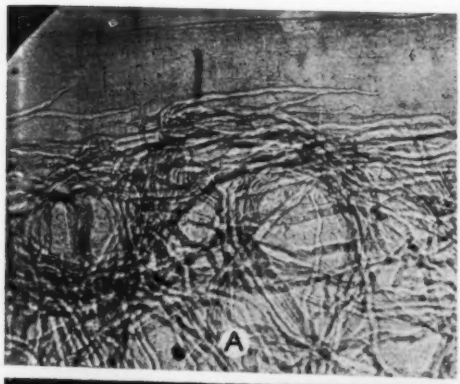
Plate XXVII

A. Margin of the leaf of *M. edule* showing intertwined arrangement of the ophiuroid sclereids. × 60.

B. Adaxial surface of the lamina of *M. Heyneanum* showing the criss-cross disposition of the ophiuroid sclereids. × 60.

C & D. Cleared leaves of *M. lushingtonii* and *Memecylon* species showing terminal sclereids of *Type II*. × 60 each.

E & F. Cleared leaves of *M. edule* and *M. malabarica* respectively showing terminal sclereids of *Type* I. E × 258; F × 60.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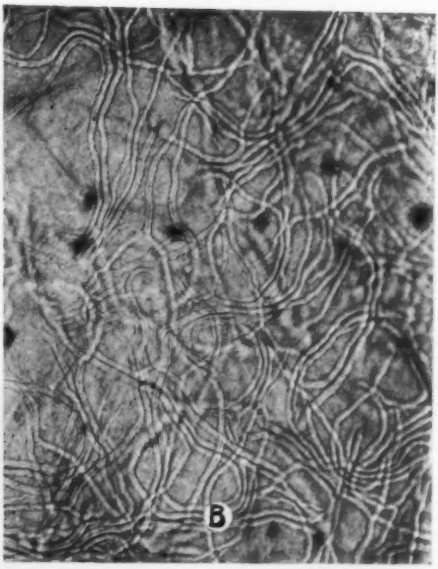
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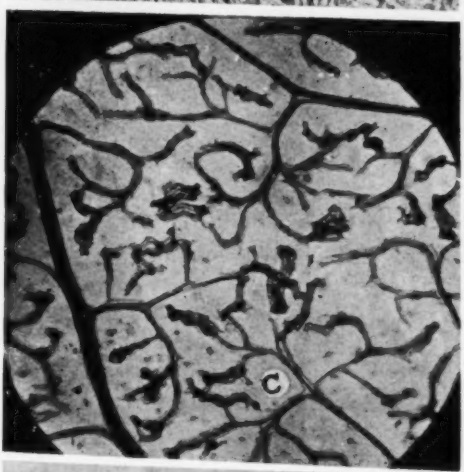
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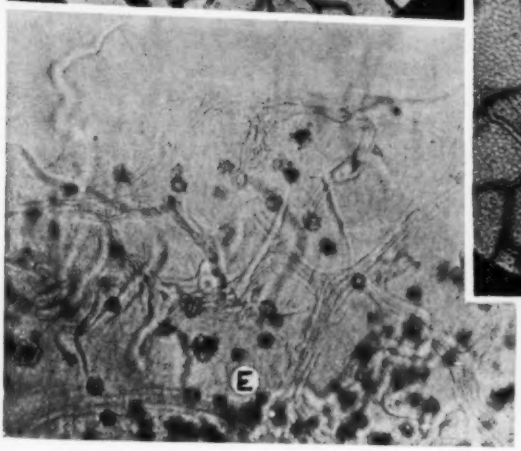
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F

STUDIES IN FISH LIVER OILS

Part II. Biological Assay of Vitamin A in Some of the Liver Oils

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It has been observed in Part I (Das, Acharya and Devadatta, 1949) that the oils extracted from some fish livers collected at different periods of the year varied in colour from pale yellow to deep red; the oils showed enormous variation in their Vitamin A content when assayed colorimetrically. They found however that there was no definite relation between the colour of the oil and its different constants.

The next problem was to study the relationship between the colour of the oil and its Vitamin A content. Ganpule and Sarangdhar (1943) state that some faint indication as to the Vitamin A potency of shark liver oil can be furnished by its colour " though this is by no means an unfailing criterion". They further remark that pale coloured or nearly colourless oils extracted from *Nebrius ferrugineus* and *Odontaspis tricuspidatus* are very poor in Vitamin A content; while oils of deep brown or reddish brown hue obtained from mottled livers are exceptionally rich. Lemon yellow, amber yellow and orange red oils are found to have a fairly high Vitamin A content. Drummond and Hilditch (1930) are of the opinion that an yellow oil is perhaps richer in Vitamin A than the one pale in colour provided every precaution has been taken to eradicate all traces of liver tissue and the accompanying enzymes.

It is not possible to specify the exact colour of the liver oils whether yellow, brown or orange, unless some device is adopted to standardise the colours. For this purpose we have devised a method to state exactly the colour of an oil in some quantitative terms. This is done as follows.—

A known quantity of the oil was dissolved in dry redistilled chloroform and the intensity of the colours (yellow and red) was noted by using a Lovibond tintometer. This method of defining the colour of the oils along with their Vitamin A content will give us a definite idea as regards the relation between the colour of the oil and its Vitamin A content. In Table I below are given the yellow, red and blue values as read on the Lovibond tintometer scale for six fish liver oils.

TABLE I

Showing the yellow and red colour values of fish liver oils with their Vitamin A (blue values) per gm. of oil

Local name of the fish liver oil	Yellow value	Red value	Colour of the oil	Blue units
Khada mushi	99·0	11·0	Reddish brown	30608
Waghbeer ..	70·0	1·0	Pale yellow	3846
Kan mushi ..	76·0	8·0	Brown	11368
Nali ..	68·0	6·0	Orange	4308
Wagli ..	98·0	1·2	Deep yellow	1728
Surmai ..	73·0	3·6	Yellowish brown	1732

From the above table it can be seen that the yellow value of the colour of an oil has no definite relation to its Vitamin A content.

But it was noticed by Das, Acharya and Devadatta (*loc. cit.*) that in the majority of cases the colorimetric assay gave more blue values for brown coloured oils than for pale yellow ones. In spite of the absence of any relationship between the colour and Vitamin A content, it was noticed, at any rate in some cases, that the deeper the colour of the oil, the more Vitamin A content it had. Investigation on Vitamin A based on colorimetry has been done by different workers in India and abroad.

Niyogi, Patwardhan and Acharya (1943), subjected the liver oils from the two fish, *viz.*, mushi (*Scoliodon Sorrokowah*) and ghol (*Sciæna miles*) to the tintometric and biological assay for Vitamin A. They used the factor 4·2 (Bomskov, 1935) to convert the blue values (as read on the Lovibond tintometer) to International Units. The two methods gave values which agreed in the case of mushi liver oil. The biological assay value for ghol liver oil was however considerably lower than that given by the tintometric method. From this observation they came to the obvious conclusion that the Vitamin A values obtained by tintometer is not a true measure. On account of cyclization of Vitamin A ghol liver oil examined by them contains a substance which does not promote growth and this substance makes up nearly a third of the chromogenic material responsible for the blue colour obtained with antimony trichloride. Therefore colorimetric method for Vitamin A is not reliable.

Embree (1939) has stated that cyclized Vitamin A, the material formed by the treatment of Vitamin A with hydrochloric acid in alcohol, has been found to occur naturally in tuna and other fish liver oils. He further states that this cyclized Vitamin A appears to have no growth-promoting power, but has considerable ultra-violet absorption at 328 μ and gives practically the same antimony trichloride reaction as Vitamin A. He calls this substance 'Spurious Vitamin A'.

As some of the liver oils examined above are already in use as substitutes for cod-liver oil, it is of utmost importance to subject them to biological assay in order to see whether the whole of the substance giving blue colour with antimony trichloride is Vitamin A or not. What is regarded as Vitamin A in these oils may, to a considerable extent, be pseudo-vitamin having no growth-promoting factor for which purpose they are used. The same six varieties of fish that were selected in Part I (*loc. cit.*) were also used in this investigation.

Experimental

Oils from the livers of fish (refer Table I) were extracted by the steaming process; Vitamin A assay in these oils was determined colorimetrically using Lovibond tintometer as described in Part I (Das, Acharya and Devadatta, *loc. cit.*) and also biologically.

Biological Estimation of Vitamin A.—Coward (1938) has stated that out of the several methods that are followed for the estimation of Vitamin A biologically, 'The increase in weight method' has the distinct advantage over the other methods in having a criterion that is easily measured. Hence, the growth method was adopted in this investigation.

The diet used in the estimation of Vitamin A:—The diet consisted of:—

Casein (extracted with ether and alcohol)	15 per cent.
Dextrinized rice starch	77 "
Marmite	4 "
Salt mixture (Osborne and Mendle, 1919)	4 "

The above diet was supplemented with diluted calciferol which provided 28 I.U. of Vitamin D_2 per rat per week, each rat being fed twice a week, 14 I.U. at a time. Starch was mixed with sufficient water to make solid lumps and these were broken into small pieces and were baked in a pan on an ordinary gas cooking stove until brittle. The starch thus dextrinized was ground and mixed in the diet. The diet was served uncooked to the rats after mixing all the ingredients.

TABLE II

Increase in weight of the albino rats in gm. for six different varieties of fish liver oils and standard β-carotene in oil

(Results are given for complete three weeks test period)

Expt. No.	Dose of fish oil mg.	Increase in wt. g.	Dose of oil in I.U. (β-carotene)	Increase in wt. g.	Vit. A in I.U. per gm. of oil biologically	Vit. A in I.U. per gm. of oil colorimetrically
		Oil No. I. Khada mushi				
I	0·086	2·6	10·5	3·9		
	0·172	5·6	21·0	8·0	82,228	1,28,554
	0·342	10·2	42·0	15·6		
II	0·129	4·2	10·5	4·1		
	0·258	8·5	21·0	8·3	83,382	1,28,554
	0·516	16·6	42·0	16·2		
		Oil No. II Waghbeer				
I	0·65	3·4	10·5	4·0		
	1·30	6·9	21·0	7·8	14,081	16,153
	2·60	14·0	42·0	15·9		
II	0·758	4·2	10·5	4·1		
	1·516	8·1	21·0	8·1	14,107	16,153
	3·032	16·5	42·0	16·0		
		Oil No, III. Kan mushi				
I	0·22	2·9	10·5	4·3		
	0·44	6·0	21·0	8·8	33,830	47,737
	0·88	11·8	42·0	17·8		
II	0·39	4·1	10·5	4·2		
	0·78	8·3	21·0	8·7	25,705	47,737
	1·56	16·5	42·0	17·6		
		Oil No. IV. Nali				
I	0·58	3·0	10·5	3·9		
	1·16	5·9	21·0	7·9	13,474	18,093
	2·32	12·1	42·0	16·7		
II	0·754	4·2	10·5	4·0		
	1·508	8·1	21·0	7·9	14,334	18,093
	3·016	16·3	42·0	16·1		
		Oil No. V. Wagli				
I	1·45	3·1	10·5	4·0		
	2·90	6·2	21·0	8·1	5,337	7,257
	5·80	12·2	42·0	15·9		
II	1·97	4·3	10·5	4·1		
	3·94	8·4	21·0	8·3	5,526	7,257
	7·88	16·9	42·0	16·0		
		Oil No. VI. Surmai				
I	1·45	3·5	10·5	3·9		
	2·90	6·9	21·0	8·0	6,357	7,274
	5·8	13·9	42·0	15·9		
II	1·66	4·2	10·5	4·0		
	3·32	8·3	21·0	8·1	6.370	7,284
	6·64	16·6	42·0	15·9		

Note :—The standard β carotene in oil used in this investigation was obtained from Nutrition Research Laboratories, Coonoor. It had been spectrographically assayed and was found to contain 2000 I.U. of Vitamin A per gram of the oil.

Procedure.—Forty-eight young rats, four weeks old and weighing between 35 gm. and 40 gm., were equally divided into six groups and fed with the Vitamin A-free basal diet mentioned above. They were weighed once a week, the first two weeks being the preparatory period, then twice a week until the rate of growth was decreased and then every day till they ceased to put on wieght. When the weight of these rats reached a steady stage three groups out of the six were given orally a supplement of 0·086, 0·172 and 0·344 mgm. of khada mushi liver oil per rat for three weeks. The animals in the other three groups, *i.e.*, the control groups received doses of standard β-carotene in oil corresponding to 3·5, 7 and 14 I.U. of Vitamin A per rat per week for three weeks (see footnote). The weights of the rats in both the groups were recorded twice a week. The results after three weeks' observation are given in Table II. The graphs showing the increase in wieght against the logarithm to the base 10 of the doses given (mgm. of mushi oil or I.U. of standard oil were drawn on the same graph). The Vitamin A content of the oil was calculated from the graph following the method adopted by Coward (*loc. cit.*) as well as by direct calculation. The amount of Vitamin A in these fish liver oils was also estimated colorimetrically. Under Table II the results of duplicate experiments are recorded, showing the doses of fish liver oil and standard β-carotene in oil administered and the resultant respective growths from which the fish liver oils are standardised. For purpose of comparison the amount of Vitamin A in International Units as obtained biologically and colorimetrically per gm. of oil are recorded in Table II. The same procedure was adopted in the case of other fish liver oils.

Discussion

In Table II are recorded the results of the analyses of six liver oils for their Vitamin A content both colorimetrically and biologically. Great variation in the Vitamin A content can be observed when the biologically obtained results are compared with those obtained by tintometric method. This is due to the fact that the Vitamin A values obtained by tintometer is not a true measure of Vitamin A because of the presence of cyclized Vitamin A in these oils which has no growth-promoting factor, but gives the same antimony trichloride reaction as Vitamin A. The conversion factor for converting Blue Units into International Units is 4·2 (Bomskov, 1935). If the colorimetric results are compared with those obtained biologically it is found that the percentage decrease in the Vitamin A content for the six liver oils, *viz.*, Khada mushi, Waghbeer, Kan mushi, Nali, Wagli and Surmai is 36·09, 12·74, 37·64, 23·15, 25·15 and 12·5 respectively. Though it is

not possible to draw a definite quantitative relationship between the yellow colour of the oil and its Vitamin A content, at this stage, it can be noticed from Table I that to some extent the decrease in the percentage of Vitamin A is accompanied by the increase in the red colour of the oil. In general, it can be confirmed that a high decrease in the percentage of Vitamin A in the oil is accompanied by an increase in the red colour of the oil.

The next problem is to find a relation between a Blue Unit and an International Unit. Norris and his colleagues (1929, 1932) investigated the relationship between the Lovibond Unit and Sherman Unit obtained biologically and found no definite relationship between the two. An attempt to establish a factor for interpreting the Carr Price value in terms of International Unit has been made by other workers also. Carr and Jewel (1933), working on a Vitamin concentrate gave a ratio of I.U./Blue Units = 21/1. Lathbury (1934) found a ratio of 20 to 1 for a distillate of the oil and 40 to 1 for cod-liver oil. Holmes and Corbet (1937), report a ratio of 30 to 1 on a crystalline Vitamin concentrate.

A similar uncertainty also exists in the interpretation of the values obtained spectrographically. The conversion factor of 1600 for blue units has been accepted by the Permanent Commission of Biological Standardisation of the League of Nations. Seshan (1940), and Rajagopal (1941) have used this factor in converting the spectrophotometric assay. The ratio of the Carr Price value to the E (extinction coefficient) values were found to vary between 10 and 38 to 1 by Seshan, the variation was however less in the case of oil having a large Vitamin A content, or when the determinations were made on the unsaponifiable matter. Rajagopal found the ratio to vary between 33 and 131. The ratio varied from sample to sample of the liver oils of shark and saw fish investigated.

In Table III below, columns 5 and 6 are given the ratio I.U. found biologically/Carr Price value on the whole oil, and I.U./Blue Unit per gram of oil respectively. It can be seen from the table that the ratio I.U./Carr Price Blue Unit varies from 68 to 92 for the six liver oils studied in this investigation. The ratio as can be seen from the table varies from oil to oil indicating that greater discrepancies are likely to be met with if a uniform conversion factor is applied to the Blue values obtained with oils from different species of fish. From the above results it can be stated that as far as possible whenever a new source of Vitamin A is found, it should be subjected to all the three methods of assay before applying any factor to convert the colorimetric or spectrophotometric values into the International Units of Vitamin A.

No doubt it will be very accurate if all the methods of assay are to be performed in order to know the exact Vitamin A content of a particular sample of liver oil. But in industry it is not possible to perform all these methods of analyses as one has to assess the cost of the oil on the spot on its Vitamin A content only. For this purpose a simple method of assay is quite essential. The ratio I.U./Blue Units per gram of the oil was found out for all the six liver oils studied, and the results are given in the last column in Table III. As can be seen from the table, the ratio varies from 2·62 to 3·67 (average being 3·16). Therefore instead of taking the Bomskov factor (*loc. cit.*), *i.e.*, 4·2 in converting the Blue values into the International Units, it will be found convenient to find a factor for the Indian fish liver oils to convert the Blue values (obtained colorimetrically) into International Units. Therefore the six varieties of fish liver oils that have been worked out for their Vitamin A assay biologically were treated statistically in order to find out a factor which could be used to convert Blue Units of an oil into International Units. The results of the biological assay of these six liver oils when treated statistically gave 3·16 as a factor which was found to be significant. Therefore it can be stated that for Indian fish liver oils factor 3 may conveniently be used in order to convert Blue values into International Units.

TABLE III

Showing the relation between International Unit, Carr Price Unit and Blue Unit per gram of the oil

1 Local name of the fish liver oil	2 I.U. biologically	3 Blue value	4 C.P. value on the whole oil	5 I.U./C.P. value	6 I.U./Blue value
Khada mushi ..	82,805	30,608	1224	68	2·71
Waghbeer ..	14,094	3,846	154	91	3·66
Kan mushi ..	29,767	11,368	455	64	2·62
Nali ..	13,904	4,308	172	82	3·18
Wagli ..	5,431	1,728	70	86	3·14
Surmai ..	6,363	1,732	70	92	3·67

SUMMARY AND CONCLUSION

1. The liver oils of six varieties of fish have been analysed for their Vitamin A content biologically as well as tintometrically. Great variation in the Vitamin A content is observed when the biologically obtained values are compared with those obtained by colorimetric method. Though it is

not possible to draw any definite relationship between the yellow colour of the oil and its Vitamin A content, it can be noticed to some extent that a decrease in the percentage of Vitamin A is accompanied by an increase in the red colour of the oil.

2. The ratio I.U./Carr Price Blue Units for the liver oils studied vary from oil to oil indicating that greater discrepancies are likely to be met with if a uniform conversion factor is applied to the Blue values obtained with oils from the livers of different species of fish. It is suggested that as far as possible, whenever a new source of Vitamin A is found it should be subjected to all the three methods of assay before applying any factor to convert the colorimetric or spectrophotometric values into the International Units of Vitamin A. For a rough estimate of Vitamin A in industry a suggestion to take the factor 3 for converting Blue values into the International Units is made. Bomskov factor 4·2 is misleading in the case of liver oils of fish in India.

ACKNOWLEDGEMENT

The authors have great pleasure in thanking Dr. V. N. Patwardhan, Director, Nutrition Research Laboratories, I.R.F.A., Coonoor, for kindly supplying reference β-carotene in oil used in the Vitamin A assay. Our thanks are also due to Dr. B. G. Acharya and Dr. B. G. S. Acharya of Garutman Laboratories for giving facilities and helpful criticism.

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LOWER EPIDERMIS OF LEAF MIDRIB AS AN INDICATOR OF ITS HARDNESS IN SUGARCANE

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I. Introduction

Issac (1939) while working on the insect-pests of sugarcane, reported that the hardness of leaf midrib was a major factor in imparting resistance to the top-borer, *Scirpophaga nivella* Fabr., and that this character could be used in breeding and selection of new varieties. Thuljaram Rao and Venkataraman (1941) confirmed these observations and reported its inheritance in sugarcane hybrids.

In view of the importance thus given to the suggestion, and the likely bearing and far-reaching effect of these findings on the future breeding programme and servicing of sugar industry in India, work was simultaneously undertaken at this station as well to critically examine the hypothesis in detail, first by evaluating the hardness of leaf midrib in terms of both its physical manifestation and anatomical structure (Khanna, 1940, 1941) and then by correlating the index of hardness with the infestation data.

Thuljaram Rao (1947) gives a method for the estimation of midrib hardness which is slow and laborious as all methods involving camera lucida drawings are, and which as such cannot be utilised to collect a large number of observations, so essential in the first place, for determining the hardness of midrib of a particular variety by subjecting the data to statistical examination, and in the second place, for the purpose in view, namely, the quick selection of seedlings, got out of a certain cross and the speedy evaluation of the role played by parents in imparting the hardness of midrib to their progeny. Because of the highly heterozygous nature of genetical constitution of genus Saccharum as a whole, the study of a large number of seedlings resulting from a cross with a view to find out the probability of their inheriting a desired character, cannot be overemphasised.

The present contribution deals with epidermis in relation to the hardness of leaf midrib as determined by a physical method.

II. Material and Methods

Thirteen varieties, namely, Co 210, Co 213, Co 285, Co 299, Co 313, Co 331, Co 421, Co 513, P.O.J. 2725 *Saraitha* and *Chin* (*Saraitha* group), *Sewari* (*Nargori* group) and *Hemja* (*Mungo* group) representing a very vide range of hardness of leaf midrib were selected for this study. Three one-inch pieces cut from the midrib of standard leaves of stalks of the same age, one from each at a distance of 3″ from the transverse mark, formed a sample for a variety. Half of each piece after removing the upper epidermis was macerated in 10% nitric acid. The lower epidermis was mounted in Canada Balsam after the usual process of washing, staining with Safranin and dehydration. Hand sections taken from the other half were also stained with Safranin and made into permanent mounts. Only two characters of the epidermis, namely the number of Silica cells per unit area and the thickness of outer wall of long cells, which were likely to impart hardness to the midrib were considered. Other components of the epidermis, such as the stomata and cork cells which were soft, and therefore, not likely to offer any resistance to the entry of a top borer larva were left out of consideration.

Silica cells were counted in 10 microscope-fields of the epidermis of each midrib, the diameter and area of the field being 470 μ, and 0·17 sq. mm. respectively. Thirty measurements of the thickness of the outer wall of the long cells in the neighbourhood of the central vascular bundle were taken from transverse sections, ten from each midrib.

The hardness of a midrib was expressed in terms of the weight required to puncture it on its convex side at 3″ from the transverse mark with a fine but blunt needle (0·6 mm. in diameter at its piercing end). The needle was fixed to a rind hardness testing apparatus designed by Puri and Venkatraman (1929) and improved upon (Plate XXVIII) by one of the authors (Khanna, 1935). Each time a 50 gm. weight was added till the midrib was punctured. To the weight actually put on the circular plate, the weight of the latter along with that of the plunger which came to 510 gm. was added. Twenty such determinations were made for each variety.

Also anatomical structure as detailed below for four varieties, Co 285, P.O.J. 2725, *Chin* and *Saraitha* (*Saraitha* group) together with that of Co 213 and Co 513 was studied to understand the erratic behaviour of the former group discovered when data on the originally selected three characters were statistically analysed:—

1. (*a*) The number and size of vascular bundles.
   (*b*) The size of sclerenchymatous cushions on which medium-sized vascular bundles were situated.

2. Thickness of sclerenchymatous cell walls.
3. Area of the midrib in transverse section.

The size of a vascular bundle was found out by measuring its radial and tangential axes and multiplying the two (Khanna and Sharma, 1947) and from each midrib five of them were measured to determine its average size for a variety. Similarly the product of the base and height gave the size of sclerenchymatous cushions which were more or less columnar in shape. In this case also, five of them were measured from each midrib. Ten measurements of thickness of two adjoining sclerenchymatous cell walls were taken from each midrib at the phloem end of the vascular sheaths situated in the middle portion of the convex surface. The area of midribs was determined from camera lucida drawings (linear magnification, 25 ×) with the help of a planimeter.

III. Experimental Results

The detailed examination of the data (Table I) showed that *Sewari* (*Nargori* group) which had almost the maximum number of Silica cells per unit area (Plate XXIX, Fig. 1) was characterised by soft midrib. On the other hand, P.O.J. 2725 and Co 299 which were not significantly different from the

Table I

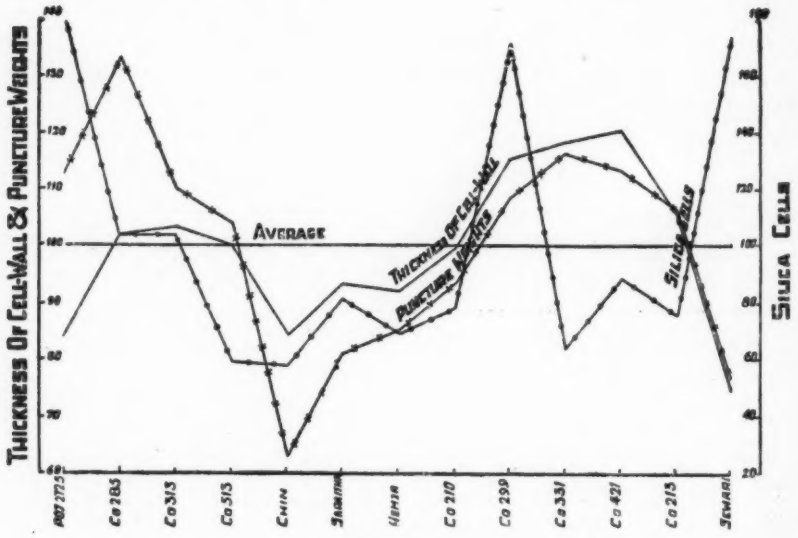
Showing number of Silica cells, thickness of outer wall of long cells of lower epidermis and midrib hardness

Variety	No. of Silica cells per unit area	Thickness of outer wall	Puncture-weights (gm.)	Percentage of the average		
	(1)	(2)	(3)	(1)	(2)	(3)
Co 210 ..	18·1	19·3	885	78	99	93
Co 213 ..	17·4	20·6	1010	75	105	106
Co 285 ..	24·0	20·0	1273	104	102	133
Co 299 ..	39·4	22·5	1033	171	115	108
Co 313 ..	24·0	20·2	1048	104	103	110
Co 331 ..	14·5	23·1	1110	63	118	116
Co 421 ..	20·3	23·5	1085	88	120	113
Co 513 ..	13·7	19·5	998	59	100	104
Saraitha ..	18·8	18·2	773	81	93	81
Chin ..	13·3	16·5	598	58	84	63
Hemia ..	16·0	18·1	810	69	92	85
Sewari ..	39·9	14·5	723	173	74	76
P.O.J. 2725 ..	41·4	16·4	1085	179	84	113
G.M. ..	23·15	19·57	955·2	..	..	..
S.E. ..	1·29	0·49	33·49	5·57	2·54	3·52
C.D. at 5% ..	3·57	1·35	94·60	..	..	..

Note.—The thickness of cell walls is given in divisions of eye-piece micrometer each of which is equal to 0·22μ.

abovementioned variety in this respect (Plate XXIX, Figs. 2 and 3) had fairly hard midribs as shown by puncture-weights. Other varieties having hard midribs such as Co 331 and Co 421 (Plate XXIX, Figs. 4 and 5) did not have per unit area, even half their number found in *Sewari*. Co 210 (Plate XXIX, Fig. 6) which was found to possess a midrib softer than that of Co 513 (Plate XXIX, Fig. 7), had significantly greater number of these cells than the latter variety. Similarly in Co 213 and Co 313 (Plate XXIX, Figs. 8 and 9) in which the midrib was softer than in Co 331, Silica cells occurred in significantly greater number per unit area than in the latter variety.

There was thus a very poor correlation between the number of Silica cells and the hardness of midrib as shown by puncture-weights (Table II, Text-Fig. 1).



Text-Fig. 1

Table II

Showing coefficients of correlation between the three characters

Character	B	C	Remarks
A ..	−0·3285	+0·1388	Not significant
B ..	..	+0·6377	Significant at 5% level

where A = No. of Silica cells.
" B = Thickness of cell walls.
" C = Puncture-weights.

Thuljaram Rao (1947) also came to a similar conclusion on the basis of his study of eight varieties. But the number of Silica cells found to occur per sq. mm. did not exceed 14 in any of the varieties selected by him for these investigations; also it was not stated how many observations he had taken for this feature. The authors on the other hand, only once out of the 390 observations recorded, came across a microscope-field (0·17 sq. mm. in area) having only 3 Silica cells in *Hemja*, or 18 per sq. mm. even for this field. The maximum number of these cells in the same field was found to be 75 in Co 299 or nearly 450 per sq. mm. The microphotographs of peelings of epidermis (Plate XXIX) cover an area of 0·68 sq. mm. approximately.

As regards the thickness of outer cell-wall of long cells, these varieties were significantly different from one another and formed more or less compact groups. Co 421, Co 331 and Co 299 had significantly thicker cell walls than the rest. Co 213, Co 313, Co 285 and Co 513 formed an intermediate group. Of the remaining six varieties, Co 210, *Saraitha* and *Hemja* formed one sub-group and *Chin*, P.O.J. 2725 and *Sewari* the other.

Thus roughly they could be grouped as follows:

(1) With hard midribs: Co 421, Co 331 and Co 299.

(2) With medium-hard midribs: Co 213, Co 313, Co 285 and Co 513.

(3) With soft midribs: (*a*) Co 210, *Saraitha* and *Hemja*.
(*b*) *Chin*, P.O.J. 2725 and *Sewari*.

On the basis of puncture-weights, the arrangement of these varieties was slightly different as given below:—

(i) With hard midribs: Co 285, Co 331, Co 421, P.O.J. 2725, Co 313 and Co 299.

(ii) With medium hard midribs: Co 213, Co 513 and Co 210.

(iii) With soft midribs: *Hemja, Saraitha, Sewari* and *Chin*.

When puncture-weights and thickness of cell walls of different varieties expressed as percentages of their respective general means (Table I), were taken into consideration it was found that in nine out of thirteen varieties, there was such a close correspondence between these two characters, that the disparity between their percentages did not exceed 7 (Text-Fig. 1). In *Saraitha* and *Chin*, the hardness of midrib was found to be 12 and 21 units less than that warranted by the thickness of their cell walls, while in the case of P.O.J. 2725 and Co 285 it was respectively 29 and 31 units harder than that arrived at, on the basis of the latter character. In spite of the erratic behaviour, on the part of these varieties which were found to be abnormal in one way or the other, when their anatomical structure was studied in this

connection, the correlation coefficient between the thickness of cell walls and the puncture-weights was found to be $+0{\cdot}6377$ which was significant at 5% level, and the former might therefore be taken as a fairly good index of the latter.

Co 285 and P.O.J. 2725 as already stated had a midrib much harder than what it ought to be on the basis of the thickness of cell walls whereas the reverse was the case with *Chin* and *Saraitha*. To understand this apparent departure from the general behaviour, their internal structure was studied in detail and compared with that of Co 213 and Co 513 which were selected not only because the disparity between the percentage values for these characters was less than 4, but also because they were all within 6% of the general mean for thirteen varieties (Table I). Co 331 and *Sewari* in which the magnitude of puncture-weights was almost equal to that of the thickness of cell wall, also could have been used for comparison. But they were far removed from the general average.

TABLE III

Showing the size and anatomical structure of midribs of six varieties

Variety	% of the average		Area of midrib sq. cm.	Vascular bundles		Size of sclerenchymatous cushions	Thickness of sclerenchymatous cell walls
	Puncture-weights	Thickness of cell walls		No.	Size		
1	2	3	4	5	6	7	8
Co 213 ..	106	105	80·5	14·0	3441	242·3	32·7
Co 513 ..	104	100	82·0	15·0	3198	252·5	38·5
Co 285 ..	133	102	55·0	15·3	2539	251·4	29·5
P.O.J. 2725 ..	113	84	68·5	10·3	3747	387·1	28·1
Saraitha ..	81	93	55·5	11·0	2820	238·2	37·1
Chin ..	63	84	38·5	10·0	2212	189·1	34·4

Note.—1. The area given in Column 4 is for a camera lucida drawing of a midrib, having a linear magnification of 25.

2. (*a*) Size of characters in Cols. 6 and 7 is given in sq. divisions of eye-piece micrometer each of which is equal to $4{\cdot}5^2$ sq. μ, and (*b*) for the thickness in Col. 8. 1 division $= 0{\cdot}22\ \mu$.

From Table III, it would appear that almost identical puncture-weights for Co 213 and Co 513 was due to the close resemblance of their anatomical structure. The larger size of vascular bundles in Co 213 was balanced by their smaller numbers, and poorer lignification of sclerenchymatous cell walls to give the same hardness to the midrib as that of Co 513 in which the vascular bundles were smaller in size but greater in number and having more

highly lignified sheaths. The sclerenchymatous cushions were more or less equal in size in both the varieties. It would thus appear that for more or less the same size of the midrib, the strengthening tissue in both the varieties was almost equal in quantity.

Now turning to defaulting varieties, one would find that in *Chin*, the midrib was smallest in area having at the same time the least amount of supporting tissue which was more or less equally lignified as that in Co 213. The number and size of both the vascular bundles and sclerenchymatous cushions were reduced by nearly 30% each, as a result of which one need not be surprised, if the puncture-weight was 21 units less than its expected value on the basis of the thickness of epidermal cell wall. Disparity between these two characters in *Saraitha* was just 12 and was easily explained when its anatomical character was compared with that of Co 513 in which the lignification of tissues was more or less of the same order as that in the variety mentioned above. The strengthening frame in *Saraitha* was much stronger than that of *Chin*. Hence although the percentage values for the thickness of cell walls in these varieties differed by only 9, those for puncture-weights had a difference of 18 points.

In the case of Co 285, there was reduction only in the size of vascular bundles and in the lignification of tissues when compared with Co 513. When judged with Co 213 as the standard, the decrease in the size of vascular bundles was enhanced whereas that in the thickness was greatly narrowed down. Slight increase in the size of sclerenchymatous cushions and in the number of vascular bundles was introduced to balance the resultant weakness in the frame, which it would thus appear, was more or less of equal strength in all the three varieties, *viz.*, Co 213, Co 285 and Co 513 as indicated by the thickness of the epidermal cell walls. But this frame in the case of Co 285 supported a midrib which was nearly $\frac{2}{3}$ the size of those of Co 213 and Co 513, and hence the puncture-weight for this variety went up by 31 units. P.O.J. 2725 had unusually large vascular bundles and sclerenchymatous cushions which were almost 50% more in size than those of Co 285. The thickness of sclerenchymatous cell walls being more or less the same in both the varieties, the effect of increase in size was offset to a very great extent by reduction in their number and increase in the area of midrib. Consequently the puncture-weight for P.O.J. 2725 was less than that of Co 285. It is interesting to note that difference between the percentages of thickness of cell wall in these two varieties was 18 as against 20 for the puncture-weights.

A fuller contribution on the method of estimation of hardness of midrib by the study of anatomical structure will follow in due course.

IV. Summary

1. (*a*) Number of Silica cells per unit area in the lower epidermis of leaf midribs of thirteen varieties of sugarcane, covering a wide range of hardness of leaf midribs was noted. P.O.J. 2725, *Sewari* (*Nargori* group) and Co 299 had on the average, 39·4 to 41·4 of them in a circular field which was 470 μ in diameter, whereas in Co 331, Co 513 and *Chin* (*Saraitha* group) the number was as low as 13·3 to 14·5 in the same area.

(*b*) Silica cells were found to have no consistent association either with the weight required to puncture the midrib on its convex side or with the thickness of the outer cell walls, both the correlation coefficients being too low to be significant even at 10% level.

2. (*a*) The range of the thickness of outer walls of long cells in the epidermis as expressed in the divisions of the eye-piece micrometer was from 14·5 in *Sewari* (*Nargori* group) to 23·5 in Co 421 (1 division = 0·22 μ).

(*b*) The varietal differences were significant at 5% level.

3. (*a*) Weights required to puncture a midrib varied from 598 gm. in *Chin* to 1273 gm. in Co 285 and these for the varieties studied were found to be significantly different at 5% level.

(*b*) The correlation coefficient between this character and the thickness of cell walls was found to be + 0·6377 which was significant at the same level.

(*c*) The thickness of outer wall of long cells of lower epidermis could, therefore, be used as a fairly reliable indicator of the puncture-weight and, therefore, of the hardness of a leaf midrib.

4. The erratic behaviour of four varieties, namely Co 285, P.O.J. 2725, *Chin* and *Saraitha* (*Saraitha* group) in which the puncture-weights were found to be higher or lower than those warranted by the thickness of cell walls was explained by the size and number of vascular bundles and the sclerenchymatous cuchions together with the thickness of sclerenchymatous cell walls when these characters were considered in relation to the size of a midrib in cross-section. All the four varieties were found to be abnormal for one character or the other.

V. Acknowledgement

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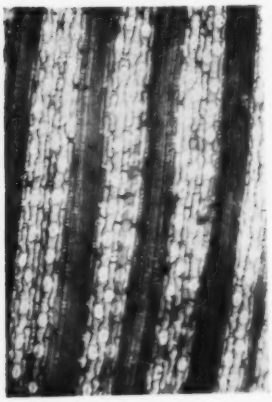

FIG. 1

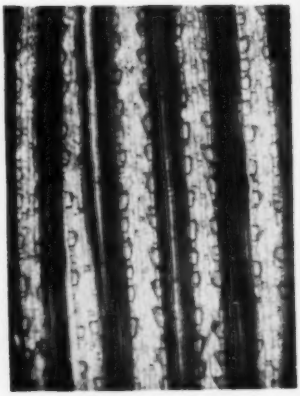

FIG. 4

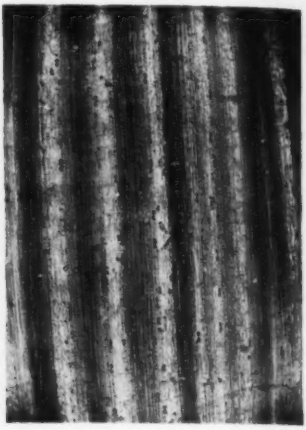

FIG. 7

FIG. 2

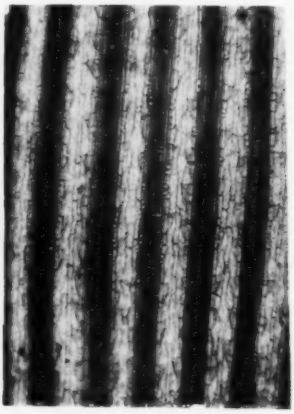

FIG. 5

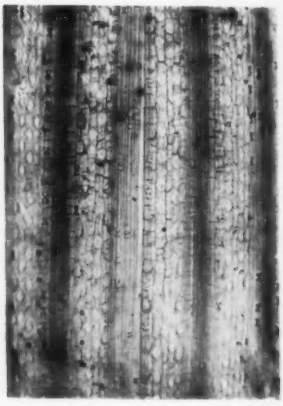

FIG. 8

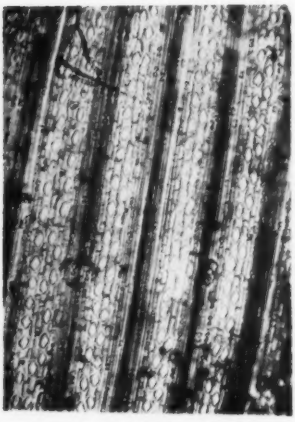

FIG. 3

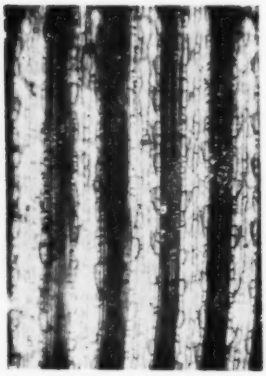

FIG. 6

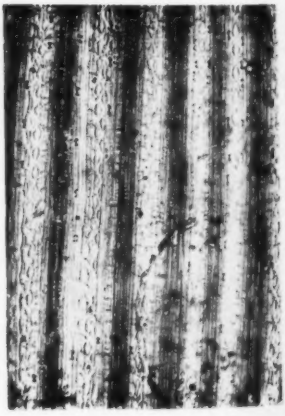

FIG. 9

VI. References

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Explanation of Plates

PLATE XXVIII

A rind hardness testing apparatus with midrib placed in position for the determination of puncture-weight. The midrib is kept on a block of hard paraffin so as to ensure that the piercing point is the same for all the midribs. If it strikes a hard surface, it will be altered in respect of its diameter and sharpness of the piercing surface, and puncture-weights will not be comparable.

PLATE XXIX

Peelings of lower epidermis of leaf midribs of sugarcane. Fig. 1, *Sewari*; Fig. 2, P.O.J. 2725; Fig. 3, Co 299; Fig. 4, Co 331; Fig. 5, Co 421; Fig. 6, Co 210; Fig. 7, Co 513; Fig. 8, Co 213; Fig. 9, Co 313; Figs. 1, 2 and 3 show a much greater density of Silica cells than Figs. 4, 5, 6, 7 and 8. Fig. 9 occupies an intermediate position in this respect. (Magnification × 90).

INFLUENCE OF VITAMIN C AND OXIDISING AGENTS ON PROTHROMBIN

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There is very little information available in literature regarding the nature of the action of vitamin C on blood coagulation and particularly on the prothrombin of the blood plasma. Bürcker (1935) found that vitamin C accelerated the coagulation of blood. Later Tislowitz (1937) and Terazawa, Takeda and Mizokuchi (1937) studied the action of vitamin C on blood coagulation *in vivo* by injecting 50 mg. of vitamin C per kilogram body weight in normal rabbits and obtained results in conformity with the findings of Bürcker. But Dyckerhoff and Pretzsch (1940) as a result of their investigations on the effect of intravenous injection of vitamin C in normal subjects on their coagulation time, reported that vitamin C had neither activating nor inhibiting effect on blood coagulation. As to the effect of heavy metals on blood coagulation Hauslar and Vogel (1931) reported that the coagulation of blood was inhibited by heavy metals like copper sulphate *in vitro* and the inhibition was attributed to the partial or complete elimination of fibrinogen by way of precipitation by the heavy metals concerned.

Till now all the work on the influence of vitamin C on blood coagulation had been carried out by using whole plasma instead of pure prothrombin. Even the heavy metal effect was tried on whole plasma.

In view of the contradictory results obtained by the above workers on the nature of the action of vitamin C on coagulation, an attempt was made to make a systematic study on the effect of vitamin C, heavy metals and their combination and oxidising agents like iodine and hydrogen peroxide on pure prothrombin.

Experimental

For the preparation of prothrombin the methods given by Seegers and his co-workers (1938, 1940) with slight modifications were used. Method (A) consists in the precipitation of prothrombin from plasma with acetic acid and subsequent denaturation of fibrinogen; while method (B) involves adsorption of prothrombin by magnesium hydroxide and its subsequent elution by phosphate or acetate buffer.

Method (*A*).—50 c.c. of plasma obtained from fresh bovine blood from the slaughter-house was used. The plasma was diluted ten times with distilled water. Then 2% acetic acid was added to bring the pH of the solution to 5·3. At this pH, all the prothrombin was precipitated along with fibrinogen and some other impurities. The solution was centrifuged and the precipitate was dissolved in 25 c.c. of M/15 phosphate buffer (pH 7·0) or M/5 acetate buffer (pH 6·0) as required. The solution was then kept at a temperature of 52° C. for 3–5 minutes, when fibrinogen and other impurities separated out. The solution was then allowed to stand overnight in the refrigerator. Most of the fibrinogen which separated on keeping was filtered off and the clear solution was used as the enzyme prothrombin.

Method (*B*).—50 c.c. of plasma was diluted 10 times with distilled water and prothrombin was precipitated by acetic acid precipitation as before. The precipitate was centrifuged off and was dissolved in 30 c.c. of 0·9% saline. The saline solution was then treated with 10 c.c. of magnesium hydroxide (15% solution in normal saline) and kept for 15 minutes at room temperature, when all the prothrombin was completely adsorbed by magnesium hydroxide. The mixture was then centrifuged and the prothrombin was eluted from the adsorbent with 25 c.c. of M/15 phosphate buffer (pH 7·0) or M/5 acetate buffer (pH 6·0) as required. Carbon dioxide was then passed through the eluate for 2–3 minutes for activating the prothrombin. The resulting clear solution was used as the pure prothrombin solution.

Preparation of Prothrombin—free plasma for use as substrate in the determination of the prothrombin time.—20 c.c. of plasma was shaken well with 2·0 c.c. of magnesium hydroxide suspended in saline solution and kept in the cold for 15 minutes. The solution was then centrifuged and the clear supernatant liquid was used as the substrate for the determination of the prothrombin time.

Determination of prothrombin time.—The reaction mixture for the determination of the activity of prothrombin consisted of 0·2 c.c. of substrate, 0·2 c.c. $CaCl_2$—*Venom mixture* [1 gm. of Russel Viper Venom (Haffkine Institute, Bombay) in 10,000 c.c. of 0·025 M/$CaCl_2$] and 0·2 c.c. of the prothrombin preparation. The time taken in seconds for this mixture to clot at 37° C. was taken as a measure of the prothrombin activity. The formation of a cobweb was taken as the clotting point.

B. D. H. sample of vitamin C was used throughout this work and all the other chemicals used were of the highest grade of purity.

To eliminate the effect of any traces of Cu, glass distilled water, distilled twice from an-all pyrex still was used in all the experiments with vitamin C.

The pH of all the solutions used in testing their influence on the activity of prothrombin, was always adjusted to the pH of the experimental solution.

The influence of vitamin C on the activity of prothrombin at pH 6·0 *and* 7·0.—The reaction mixtures contained 5 c.c. of prothrombin (prepared by method A) in the buffer of the required pH, and 5 c.c. of buffer or solution of vitamin C (5 mg.) in buffer, whose influence on the enzyme was to be studied. M/5 acetate and M/15 phosphate buffers were used for pH 6·0 and pH 7·0 respectively. The reaction mixtures were incubated at 25° C. and at stated intervals of time, the prothrombin time was determined at 37° C. The results are presented in Table I.

TABLE I. *Influence of Vitamin C on Prothrombin*

	Prothrombin time in seconds after incubation					
	at pH 6·0 for			at pH 7·0 for		
	0 min.	30 min.	60 min.	0 min.	30 min.	60 min.
Prothrombin alone ..	85	88	90	70	77	80
Prothrombin + Vitamin C ..	80	88	88	65	75	80

The results indicate that vitamin C alone has no effect on prothrombin activity at pH 6·0 and 7·0.

Effect of copper on prothrombin at pH 7·0.—The reaction mixture consisted of 5 c.c. of prothrombin solution in M/15 phosphate buffer at pH 7·0 (prepared by method A) and buffer or solution of copper sulphate of the respective concentrations, the total volume being adjusted to 10 c.c. in each case. The reaction mixtures were incubated at 25° C. and at stated intervals of time the prothrombin time was determined at 37° C. The results are presented in Table II.

TABLE II. *The Influence of Cu on Prothrombin at pH* 7·0

Experimental mixtures	Prothrombin time in seconds after incubation for		
	0 min.	30 min.	60 min.
Prothrombin alone ..	65	76	80
Prothrombin + Cu^{++} (1·0γ) ..	64	70	80
Prothrombin + Cu^{++} (2·5γ) ..	65	75	77
Prothrombin + Cu^{++} (5·0γ) ..	63	77	75
Prothrombin + Cu^{++} (10·7γ) ..	66	75	77

The results show that copper alone, even at higher concentrations has no effect on the activity of prothrombin.

Effect of varying concentrations of copper on prothrombin at pH 6·0.—The prothrombin was prepared by Method A. The reaction mixture consisted of 5 c.c. of prothrombin solution, and M/5 acetate buffer (pH 5·0) or solution of copper sulphate of the particular concentration and the total volume was made up to 10 c.c. in each case. The reaction mixtures were incubated 25° C. and at regular intervals of time, the prothrombin time was determined at 37° C. The results are presented in Table III.

TABLE III. *Effect of Cu on Prothrombin at pH* 6·0

Experimental mixtures	Prothrombin time in seconds after incubation for		
	0 min.	30 min.	60 min.
Prothrombin alone ..	86	88	90
Prothrombin + Cu (5·3γ) ..	80	92	90
Prothrombin + Cu (10·7γ) ..	89	99	92
Prothrombin + Cu (21·4γ) ..	88	20	95

The results clearly show that copper even at such a high concentration as 21·4 per 10 c.c. does not affect the activity of prothrombin.

Effect of vitamin C—Cu complex on prothrombin activity.—The effect of vitamin C—Cu complex on prothrombin was next investigated. Prothrombin prepared by both the methods at both pH values 6·0 and 7·0 was used.

The reaction mixtres contained 5 c.c. of prothrombin, 2 c.c. of vitamin C solution, containing 5 mg. of vitamin C, 1 c.c. of copper sulphate solution containing 10·7 γ Cu and buffer to make up the total volume to 10 c.c. The reaction mixtures were incubated at 25° C. and at stated intervals of time, the prothrombin time was determined at 37° C. The results are presented in Table IV.

The results clearly indicate that vitamin C—Cu complex also does not affect the activity of prothrombin. The absence of any inhibition of the prothrombin activity by vitamin C alone or vitamin C—Cu complex points to the possibility of the presence of a powerful protective factor in the prothrombin preparation, which prevents the oxidation of vitamin C in presence and absence of copper.

TABLE IV. *The Influence of Vitamin C, Copper and Vitamin C—Copper Complex on the Activity of Prothrombin*

Experimental mixtures	Prothrombin time in seconds											
	Prothombin prepared by Method A						Prothrombin prepared by Method B					
	at pH 6·0 after incubation for			at pH 7·0 after incubation for			at pH 6·0 after incubation for			at pH 7·0 after incubation for		
	0 min.	30 min.	60 min.	0 min.	30 min.	60 min.	0 min.	30 min.	60 min.	0 min.	30 min.	60 min.
Prothrombin alone	86	88	90	96	100	110	50	55	65	65	75	80
Prothrombin + Cu	90	90	95	95	100	115	50	60	65	65	75	75
Prothrombin + Vitamin C	80	88	88	98	100	100	50	55	60	65	70	70
Prothrombin + Copper + Vitamin C	82	92	98	100	105	120	55	60	70	70	75	85

So the correlation between vitamin C oxidation and the activity of prothrombin in presence and absence of vitamin C alone and vitamin C—Cu complex was next studied.

Correlation between Vitamin C oxidation and the activity of Prothrombin.— The prothrombin was prepared by Method A in M/5 acetate buffer pH (6·0) and the following reaction mixtures were set up containing:—

1. 5 c.c. prothrombin + 5 c.c. M/5 acetate buffer (pH 6·0).
2. 8 c.c. M/5 acetate buffer (pH 6·0) + 2 c.c. vitamin C (5 mg.).
3. 5 c.c. prothrombin + 2 c.c. Vitamin C (5 mg.) + 3 c.c. M/5 acetate buffer (pH 6·0).
4. 7 c.c. M/5 acetate buffer (pH 6·0) + 2 c.c. vitamin C (5 mg.) + 1 c.c. $CuSO_4$, $5H_2O$ solution (10·7 γ Cu).
5. 5 c.c. Prothrombin + 2 c.c. vitamin C (5 mg.) + 1 c.c. $CuSO_4$, $5H_2O$ solution (10·7 γ Cu).

All the mixtures were incubated at 25° C. and the prothrombin time was determined at stated intervals of time. Simultaneously, the vitamin C content was also determined in 1 c.c. of the reaction mixtures by titration with 2:6 dichlorophenol indophenol. The results are presented in Table V.

The results indicate that vitamin C is stabilized to a great extent both in presence and absence of copper by prothrombin. There is very little oxidation in the case of vitamin C—Cu complex in the presence of prothrombin. But that may not be sufficient to effect any considerably inhibition

TABLE V. *Prothrombin activity and Vitamin C Oxidation*

Experimental mixtures	Prothrombin time in seconds and vitamin C content after incubation for					
	0 minutes		30 minutes		60 minutes	
	Prothrombin activity	Vitamin C content mg.	Prothrombin activity	Vitamin C content mg.	Prothrombin activity	Vitamin C. content mg
Prothromdin alone ..	85	..	90	..	96	..
Vitamin C alone ..	..	5	..	4·2	..	4·2
Prothrombin + Vitamin C ..	86	5	96	4·9	96	4·8
Vitamin C + Cu ..	..	5	..	3·4	..	2·5
Prothrombin + Vitamin C + Cu	85	5	100	4·6	100	4·6

of the enzyme activity. The results clearly show the presence of a powerful protective factor in prothrombin which annuls the oxidation of vitamin C in presence and absence of copper. The lack of any inhibiting effect of vitamin C—Cu complex on prothrombin is due to the vitamin being stabilised by the protective factor present in the prothrombin.

The prothrombin preparation itself when treated with a solution of sodium-diethyl-dithio-carbamate gave a slight yellow colour. So it was thought that traces of copper might be present in prothrombin solution and the effect of sodium-diethyl-dithio-carbamate on prothrombin was investigated to find out whether copper was in any way concerned with the activity of prothrombin.

Effect of sodium-diethyl-dithio-carbamate on prothrombin.—Prothrombin was prepared by Method A and was taken up in M/5 acetate buffer (pH 6·0).

The reaction mixtures consisted of 5 c.c. of prothrombin and 5 c.c. of M/5 acetate buffer (pH 6·0) or solution of sodium-diethyl-dithio-carbamate containing 5 mg. of the substance, whose influence on the prothrombin was to be determined. The mixtures were incubated at 25° C. and at regular intervals of time, the prothrombin was determined at 37° C. The results are presented in Table VI.

The results show that the prothrombic activity is slightly inhibited by sodium-diethyl-dithio-carbamate. The results, however, do not warrant any definite conclusion regarding the role of copper in regulating the activity of prothrombin. Further work is necessary. To study the influence of oxidation on the activity of prothrombin in general, the effect of well-known oxidsing agents on prothrombic activity was next investigated.

TABLE VI. *The Influence of Sodium-diethyl-dithio-carbamate on Prothrombin Activity*

Time of incubation in minutes	Prothrombin time in seconds.	
	Prothrombin alone	Prothrombin + Sodium-diethyl-dithio-carbamate
0	100	120
30	105	130
60	105	140

Influence of iodine and hydrogen peroxide on prothrombin.—The enzyme was prepared by Method A at pH 6·0. The following reaction mixtures were set up containing:—

1. 5 c.c. prothrombin + 5 c.c. M/5 acetate buffer (pH 6·0).
2. 5 c.c. prothrombin + 2 c.c. N/100 I_2 + 3 c.c. M/5 acetate buffer (pH 6·0).
3. 5 c.c. prothrombin + 2 c.c. H_2O_2 (12 volumes) + 3 c.c. M/5 acetate buffer (pH 6·0).

The mixtures were incubated at 25° C. and at stated intervals of time the prothrombin activity was determined at 37° C.

In the case of reaction mixture (2), on addition of iodine, a heavy precipitate appeared slowly and, gradually the colour of iodine faded away. After one hour, most of the colour of iodine was destroyed showing that iodine was reduced. The results are presented in Table VII.

TABLE VII. *The Effect of Iodine and Hydrogen Peroxide on Prothrombin*

Time of incubation in minutes	Prothrombin time in seconds.		
	Prothrombin alone	Prothrombin + Iodine	Prothrombin + Hydrogen peroxide
0	117	600	200
30	200	800	200
60	200	200*	220

* The prothrombin solution containing the precipitate was filtered; the filtrate was completely free from iodine coloration.

When the prothrombin time was determined with the iodine-treated prothrombin solution without removing the precipitate, caused by addition

of iodine, the colour of the iodine disappeared when the prothrombin solution was mixed with the plasma substrate. The clots formed, in the cases of iodine and hydrogen peroxide-treated prothrombin, were flimsy and transparent. Hydrogen peroxide in the concentration used above did not give any precipitate with the prothrombin solution.

In a recent paper Jaques (1943) reported that iodine and hydrogen peroxide were reduced by fibrinogen and that iodine was found to give a heavy precipitate with fibrinogen, and that this behaviour of iodine with fibrinogen was specific, as none of the other serum proteins were found to react with iodine in such a manner.

In the light of Jaques' findings, the gradual reduction of the colour of iodine in the presence of prothrombin and the formation of a heavy precipitate might be due to the presence of fibrinogen as a contaminant in the prothrombin preparations. Similarly, the disappearance of the colour of iodine when the iodine-treated prothrombin solution was mixed with the plasma substrate might be due to the action of the fibrinogen present in the substrate. After sixty minutes incubation with iodine, all the iodine was reduced and the filtrate showed normal prothrombin activity, while at zero time and after thirty minutes' incubation, the unfiltered solutions of the iodine-treated prothrombin lost most of their activity. This is really an apparent loss and the discrepancy can be explained in the following manner. In the first two determinations, *i.e.*, at zero time and after 30 minutes' incubation, tnere is some excess iodine (unreduced) with the prothrombin solution and on adding it to the plasma substrate, the fibrinogen of the substrate is removed from the field of action by it, and thus the clotting is delayed. In the third case after 60 minutes incubation, all the iodine is reduced by the fibrinogen present along with the prothrombin solution and there is no free iodine to react with the prothrombin molecule or the fibrinogen of the plasma substrate and hence the absence of any delay in clotting.

The results in Table VII show that hydrogen peroxide at the concentration used does not have any pronounced effect on the prothrombin activity.

Influence of varying concentrations of iodine on Prothrombin activity.— The prothrombin was prepared by Method A in M/5 acetate buffer at pH 6·0.

The reaction mixtures contained 5 c.c. of prothrombin solution, varying amounts of N/10 iodine ranging from 0·1 c.c. to 2·0 c.c. and M/5 acetate buffer (pH 6·0) to make up the total volume of the reaction mixture to 8 c.c. The mixtures were incubated at 25° C. for 30 minutes and filtered. The prothrombin times of the filtrate in each case was determined at 37° C. as shown in Table VIII.

TABLE VIII. *Effect of Concentration of Iodine on Prothrombin*

	Amount of N/10 Iodine added in c.c.	Prothrombin time in seconds
Prothrombin alone ..	..	100
Prothrombin + Iodine ..	0·1	105
do ..	0·25	400
do ..	0·50	480
do ..	1·0	750
do ..	2·0	>900

The above results indicate that there is a regular increase in the inactivation of prothrombin with the increase in the concentration of iodine. This inactivation may be due to the action of the excess iodine on the fibrinogen of the plasma substrate or to its action directly on the prothrombin molecule itself. This can further be clarified by determining the activity of the iodine-treated prothrombin after removing the excess iodine by the use of reagents capable of removing the iodine without having any appreciable inactivating effect on the prothrombin molecule.

It is well known that sodium thiosulphate is an efficient reagent for removal of iodine. So its effect on prothrombin was investigated. Even in very low concentrations it inactivated the prothrombin and carbon dioxide is found to regenerate the activity of the prothrombin as shown by the results presented in Table IX.

TABLE IX. *Influence of Sodium Thiosulphate on Prothrombin and the Effect of CO_2 on the Thiosulphate-treated Prothrombin*

	Prothrombin time in seconds after incubation for	
	0 min.	30 min.
Prothrombin alone ..	100	100
do (after passing CO_2) ..	95	95
Prothrombin + 0·1 c.c. N/100 $Na_2S_2O_3$	395	900
do (after passing CO_2) ..	90	90
Prothrombin + 0·5 c.c. N/100 $Na_2S_2O_3$	900	>1800
do (after passing CO_2) ..	90	90
Prothrombin + 1·0 c.c. N/100 $Na_2S_2O_3$	>1800	>1800
do (after passing CO_2) ..	90	90

The enzyme was prepared by Method B at pH 6·0. The reaction mixture contained 5 c.c. of prothrombin, varying amounts of N/100 sodium thio-sulphate solution and M/5 acetate buffer (pH 6·0) to make up the total volume of the reaction mixtures to 6 c.c. The mixtures were incubated at

25° C. and the prothrombin time was determined at 37° C. at stated intervals of time.

The results show that even at very low concentrations, sodium thiosulphate inactivates prothrombin and that the inhibition is almost instantaneous. The mechanism of the complete regeneration of the activity of the inactive prothrombin by CO_2 is not known and further work is to be done for its elucidation.

Next, the organic solvents chloroform and carbon tetrachloride, which are mostly used for extracting free iodine were tested for their effect on the prothrombin activity. The results are presented in Table X.

TABLE X. *Effect of Carbon Tetrachloride and Chloroform on Prothrombin*

	Prothrombin time in seconds					
	Prothrombin prepared by Method A			Prothrombin prepared by Method B		
	Incubation time			Incubation time		
	0 min.	30 min.	60 min.	0 min.	30 min.	60 min.
Prothrombin alone ..	145	150	155	100	104	108
Prothrombin + Carbon tetrachloride	150	155	160	100	105	110
Prothrombin + Chloroform ..	150	155	160	105	105	110

The prothrombin was prepared by both the methods (A and B) in M/5 acetate buffer (pH 6·0). The reaction mixture consisted of 5 c.c. of prothrombin, 1 c.c. of M/5 acetate buffer (pH 6·0) and 2 c.c. of carbon tetrachloride or chloroform as the case may be. The total volume of the reaction mixture was always adjusted to 8 c.c. The mixtures were incubated at 25° C. and the activity of prothrombin was determined at stated intervals of time at 37° C.

The results show that both carbon tetrachloride and chloroform have no effect on prothrombin activity. These reagents can therefore be used for extracting the excess iodine from the iodine-treated prothrombin solutions without in any way affecting the activity of prothrombin.

To find out whether iodine directly affects the prothrombin activity or not, 10 c.c. of prothrombin (prepared by Method B at pH 6·0) was treated with excess iodine (1 c.c. of N/10 iodine) and kept in the refrigerator

for 30 minutes. The solution was then filtered and the excess iodine was removed by extracting it with the minimum quantity of chloroform or carbon tetrachloride. The clear colourless solution thus obtained was then tested for its activity. The results are presented in Table XI.

TABLE XI. *The Activity of Iodine-treated Prothrombin after Removal of the excess iodine with CCl_4 and $CHCl_3$*

Reaction mixtures	Prothrombin time in seconds
Prothrombin alone ..	120
Prothrombin + CCl_4 ..	125
Prothrombin + $CHCl_3$ ..	120
Prothrombin + Iodine (without removing excess iodine) ..	>900
Iodine-treated Prothrombin (after removing excess of iodine by $CHCl_3$)	>900
Iodine-treated Prothrombin (after removing excess of iodine by CCl_4)	>900

It is clear from the above results that iodine-treated prothrombin is inactive even after the removal of excess iodine by reagents which do not in any way effect the activity of prothrombin. So this rules out the possibility that iodine produces inhibition in clotting by affecting the fibrinogen of the plasma substrate and indicates that iodine may directly react with the prothrombin molecule. It may be that the prothrombin is inactivated by iodine after all the accompanying fibrinogen is oxidized by it. Indirectly the fibrinogen which is present as contaminant in the prothrombin solution protects to some extent the inactivation of prothrombin by iodine.

Next the effect of reducing agents and carbon dioxide on the regeneration of the activity of the iodine-treated enzyme was carried out.

Influence of carbon dioxide and sodium sulphite on the inactive prothrombin obtained by iodine treatment.—The prothrombin used was prepared by Method B in M/5 acetate buffer (pH 6·0). The inactive prothrombin was obtained as before by treating the enzyme with excess iodine and removing the excess iodine by carbon tetrachloride. The following reaction mixtures were set up containing:—

1. 5 c.c. prothrombin + 1 c.c. M/5 acetate buffer (pH 6·0).
2. 5 c.c. Iodine-treated prothrombin + 1 c.c. M/5 acetate buffer (pH 6·0).
3. 5 c.c. Iodine-treated prothrombin + 0·5 c.c. sodium sulphite (12%) + 0·5 c.c. M/5 acetate buffer (pH 6·0).
4. 5 c.c. Iodine-treated prothrombin + 1 c.c. M/5 acetate buffer (pH 6·0) and CO_2 passed for 1 minute.

The mixtures were kept at 25° C. for 30 minutes and the prothrombin time was determined at 37° C. The results are shown in Table XII.

TABLE XII. *Effect of CO_2 and Na_2SO_3 on the Inactive Prothrombin obtained by Iodine Treatment*

	Prothrombin time in seconds
Original prothrombin ..	40
Iodine-treated prothrombin ..	>900
do + Na_2SO_3 ..	>900
do + CO_2 ..	45

It is evident from the above results that sodium sulphite has no reactivating effect on prothrombin while carbon dioxide completely regenerates the prothrombic activity.

TABLE XIII. *Stability of Prothrombin Activated by Carbon Dioxide after Treatment with Iodine*

	Prothrombin time in seconds after being kept in the refrigerator for		
	0 min.	6 hrs.	24 hrs.
Untreated prothrombin ..	60	65	150
Prothrombin (iodine treated and reactivated by CO_2) ..	80	1800*	1800*

* When carbon dioxide was passed in this prothrombin solution, the prothrombin activity was restored to the original 80 seconds.

The prothrombin was prepared by method B at pH 6·0 and the inactive enzyme was prepared by iodine treatment as described before.

5 c.c. each of prothrombin (*a*) untreated with iodine and (*b*) treated with iodine and reactivated by carbon dioxide were kept in the refrigerator and the prothrombin activity was determined at stated intervals of time at 37° C.

The results show that the iodine-treated prothrombin reactivated by passing carbon dioxide is very unstable while the original prothrombin solution retains most of its activity even after keeping for 24 hours. The prothrombin obtained after iodine treatment and reactivation with carbon dioxide is much purer than the original enzyme in that it is completely free from fibrinogen, which is usually adsorbed along with prothrombin in negligible amounts and is a most difficult contaminant to remove. The

above results suggest the possibility that the presence of a small quantity or traces of fibrinogen may be necessary for the stability of the enzyme prothrombin.

Discussion

The results reported in the previous pages show that vitamin C has no effect on the activity of prothrombin *in vitro* and also that the prothrombin is unaffected by copper alone or vitamin C—Cu complex. Experiments with regard to the study of the relationship between the activity of prothrombin and the oxidation of vitamin C indicate that prothrombin exerts powerful protection against the non-cupric and cupric ion oxidation of vitamin C even when copper is used in such high concentration as 21·0 γ. The exact nature of the protective factor in prothrombin responsible for the stabilization of the vitamin is not clearly known. Previous experiments regarding the influence of vitamin C on amylase and urease definitely show that the inactivation of the enzymes is related to the oxidation of the vitamin. In view of the above findings, the lack of any inhibition of the prothrombic activity by vitamin C even in the presence of high concentrations of copper is due to the stabilization of the vitamin by the protective factors associated with the prothrombin.

Hauslar and Vogel (1936) reported that the coagulation of blood was inhibited by heavy metals like copper on the basis of experiments *in vitro* with whole plasma. The inhibition, according to them, was due to partial or complete elimination of fibrinogen by way of precipitation by the heavy metal concerned. While studying the effect of copper on prothrombin during the present investigation, the prothrombin solution was found to give a precipitate with copper, whenever fibrinogen was not completely removed from it by heat denaturation. But prothrombin solutions prepared by the adsorption method never developed any precipitate even when incubated with copper for long periods. Probably the complex formation between copper and fibrinogen might not be very specific when the fibrinogen is present in small amounts or traces.

The effect of oxidizing agents on prothrombin time was next studied so as to find out the effect of oxidation in general on the prothrombic activity. Among the oxidizing agents studied, iodine was found to have a marked inhibitory effect on prothrombin, the inhibition being increased with increasing concentrations of iodine. On the other hand hydrogen peroxide at the concentration used showed neither activating nor inhibiting effect on prothrombin.

When iodine was added to prothrombin solution, the colour of the iodine began to disappear, until a sufficient concentration of iodine was

reached. The solution after standing for some time developed a heavy precipitate, which according to Jaques (1943) was due to the specific reaction of iodine with the fibrinogen associated with the enzyme. The marked inactivation of prothrombin by iodine was first thought to be due to the elimination of fibrinogen from the plasma substrate by the excess iodine present in the prothrombin solution added to the clotting mixture, and not to the direct action of iodine on the prothrombin molecule. But when the excess of iodine was completely removed by extraction with organic solvents like chloroform and carbon tetrachloride, which were found to have no effect on the prothrombin solution the resulting prothrombin solution showed no activity showing thereby that iodine directly affects the prothrombin in the absence of fibrinogen. The activity of the iodine-treated prothrombin was found to be completely regenerated by passing carbon dioxide through the solution of the enzyme. Reducing agents like sodium sulphite, however, did not reactivate the inactive prothrombin obtained by iodine treatment.

During the experiments carried out for finding substances which will remove the excess iodine in the iodine-treated prothrombin without impairing the prothrombin activity, sodium thiosulphate showed marked inhibitory effect on prothrombin time and the inactive prothrombin thus obtained could be reactivated by passing carbon dioxide.

Recently Quick (1942) reported that the " component A " of prothrombin is involved in the activation or inhibition phenomena observed by the different workers when carbon dioxide was passed through prothrombin solution. In view of the above finding, the observation that inactive prothrombin solutions obtained by treatment with I_2, and $Na_2S_2O_3$ can be reactivated completely only by carbon dioxide may throw some light on the mechanism of the inactivation of prothrombin by the reagents concerned—namely, that iodine and sodium thiosulphate inactivate prothrombin by attacking the same group " component A " of the prothrombin molecule.

Though, as seen from the above observations, carbon dioxide seems to have a specific role in activating prothrombin solutions rendered inactive by a variety of substances, the exact mechanism of activation is still obscure. This really offers a very interesting field for further research.

By removing traces of fibrinogen by treatment with iodine followed by the removal of excess iodine by carbon tetrachloride and activation of the prothrombin by carbon dioxide, a very pure prothrombin was prepared. The enzyme, though quite potent and free from traces of fibrinogen as impurity, was found to be very unstable and lost most of its activity when

kept over long periods. On the other hand, under similar conditions, the prothrombin solution prepared by Method B and containing traces of fibrinogen, retained most of its activity, thereby suggesting that the presence of a small quantity or traces of fibrinogen may be necessary for the stability of the prothrombin.

The results reported in the present investigation though not very comprehensive, have an important significance, in that they throw light on the nature of prothrombin and the mechanism of blood coagulation.

Summary

Vitamin C, copper and vitamin C—Cu complex had no inhibitory effect on prothrombin activity, the lack of inhibition being due to the stabilization of the vitamin by the protective factors associated with the enzyme.

While hydrogen peroxide had no effect on prothrombin activity, iodine and sodium thiosulphate were found to inactivate prothrombin, the activity in both the cases being regenerated by passing carbon dioxide. Reducing agents like sodium sulphite, however, did not regenerate the prothrombic activity lost by treatment with iodine.

The organic solvents, chloroform and carbon tetrachloride were found to have neither activating nor inhibiting effect on prothrombin.

The presence of traces of fibrinogen along with prothrombin appears to be necessary for the stability of the enzyme.

The results have an important significance in throwing new light on the nature of the prothrombin and the mechanism of blood coagulation.

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VITAMIN C CONTENT OF LUCERNE AND THE FACTORS CONTROLLING ITS STABILITY

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Although much work has been done regarding the mineral content and the vitamin A, D and K contents of lucerne or alfa alfa (*Medicago Sativa*) very little has been reported about its antiscorbutic value. It was Levy and Fox (1935) who first reported that lucerne was a very good source of vitamin C, the concentration in the average leaf examined being about four times that found in citrus juice. Later, Marston, Quinlan-Watson and Dewey (1943) studying the dehydration of lucerne and its potentialities as a concentrated source of vitamin C, showed that lucerne leaves contained 1,000 to 1,200 mg. ascorbic acid per 100 gms. on dry weight basis. They found that lucerne did not lose much of its ascorbic acid on dehydration and the slight destruction during drying had been attributed to the presence of a powerful oxidase system in the fresh leaves.

In the following experiments, the vitamin C content of lucerne had been determined by the usual dye titration method as well as by Lugg's formaldehyde method (1942). The factors controlling the stability of vitamin C in lucerne were studied in detail with a view to examine the mechanism of the stabilisation of the vitamin in plant material.

Experimental

Preparation of 2:6 *dichlorophenol-indophenol reagent.*—A 0·5% solution of the dye was prepared in distilled water buffered at pH 7·2 with M/15 phosphate buffer.

The dye was always standardised against N/100 sodium thio-sulphate and 1 c.c. of the dye is equivalent to 0·264 mg. vitamin C.

Vitamin C content of Lucerne as determined by (*a*) *Dye-titration method and* (*b*) *Lugg's formaldehyde method.*—

(*a*) *Dye-titration method.*—Two grammes of fresh lucerne leaves were ground well with 50 c.c. of 5% metaphosphoric acid along with some glass powder to facilitate grinding. The whole material was then filtered. The

vitamin C was estimated by titrating a 5 c.c. aliquot of the clear metaphosphoric acid extract after adding 0·5 c.c. glacial acetic acid with 2: 6 dichlorophenol-indophenol. The appearance of a pink colour stable for 30 to 60 seconds was taken as the end point.

From the amount of dye consumed, the vitamin C was calculated, taking that 1 c.c. of the dye corresponds to 0·264 mg. of vitamin C.

Vitamin C content per gramme of fresh lucerne as judged by the usual-dye titration method .. .. 1·78 mg.

(*b*) *Lugg's Formaldehyde method.*—Lugg's method as modified by Mapson (1943) was adopted. The method is based on the principle that formaldehyde at pH 2·0 specifically condenses with ascorbic acid in the presence of all other reducing substances, the complex thus formed being unreactive towards the dye.

2 gms. of fresh lucerne leaves were extracted with 50 c.c. of 5% metaphosphoric acid as before. 5 c.c. of the clear extract was then titrated with the dye after bringing the pH of the extract to 1·2 by adding the requisite quantity of glacial acetic acid. The total amount of the dye required for 50 c.c. of the extract was then calculated and that value was termed A. 42·8 c.c. of the extract were then brought to pH 2·0 by adding 1·2 c.c. of citrate buffer prepared according to Lugg (1942). The final extract was then condensed with 11 c.c. of 40% formalin so that the final concentration of formaldehyde in the total mixture was 8%. At stated intervals of time, 5 c.c. aliquots were removed and titrated with the dye as given in method (*a*). The condensation was allowed to take place till the 5 c.c. aliquot gave a constant titre. From the final titre, the amount of dye required for the original 50 c.c. extract was calculated and the value was termed as B. A-B gives the true vitamin C content.

Results.—

Amount of dye required for 50 c.c. extract at pH 1·2 13·5 c.c.—A

Amount of dye required for 50 c.c. original extract after condensation with formaldehyde at pH 2·0 0·0 c.c.—B

Amount of dye representing the true vitamin C in lucerne .. .. 13·5 c.c. (A–B)

Taking that 1 c.c. of the dye corresponds to 0·264 mg. of vitamin C,

Vitamin C content per gm. of fresh lucerne as judged by Lugg's formaldehyde method. .. 1·78 mg.

The results show that all the reducing substance present in lucerne is true vitamin C.

Stability of vitamin C in the lucerne juice.—The juice was extracted from the lucerne leaves by grinding the leaves with fine sand and metaphosphoric acid and squeezing the ground material through a muslin cloth. The filtered juice was kept in a 100 c.c. beaker in the refrigerator and at stated intervals of time ranging over a period of 24 hours, the vitamin C content of the juice was determined in 2 c.c. aliquots by titrating the metaphosphoric acid extract of the juice with the dye. The results are presented in Table I.

TABLE I

Time of incubation in hours	Mg. of Vitamin C. in 2 c.c. of the lucerne juice
0	1·0
1	0·84
2	0·78
6	0·70
12	0·30
24	0·0

The results show that there is a gradual loss of the vitamin on standing and that the vitamin is completely lost after keeping the juice for 24 hours. This points to the possibility of the presence of an oxidase system in lucerne leaves.

Experiments were then carried out to find whether lucerne contained any potent ascorbic acid oxidase. The oxidation of pure vitamin C in the presence and absence of fresh and dialysed lucerne juice was studied.

Preparation of the dialysed lucerne juice.—The fresh lucerne leaves were squeezed through a cloth and the fresh juice that was obtained was dialysed for 20 hours at 25° C. against distilled water in collodion bags with sufficient toluene at the top. The juice in the bag was then centrifuged and the clear supernatant liquid was used as the dialysed lucerne juice.

Oxidation of vitamin C in presence and absence of fresh dialysed and undialysed lucerne juice at pH 5·6.—The reaction mixture contained 10 c.c. of M/5 acetate buffer (pH 5·6), 5 mg. of vitamin C, 2 c.c. of the undialysed or the dialysed lucerne juice as the case may be and distilled water to adjust the total volume of the mixture to 15 c.c. The mixtures were incubated at 30° C. and at stated intervals of time, the vitamin C was estimated in aliquots of the mixture by titration with the dye. The results are summarized in Table II.

TABLE II

The oxidation of Vitamin C in presence and absence of undialysed and dialysed lucerne juice at pH 5·6

	Mg. of Vitamin C in the total volume of the reaction mixture after incubation for			
	0 min.	30 min.	60 min.	120 min.
Vitamin C alone ..	5·0	4·0	3·2	2·4
Vitamin C + 2 c.c. undialysed lucerne juice ..	5·0	4·8	4·8	4·6
Vitamin C + 2 c.c. dialysed lucerne juice ..	5·0	3·8	2·8	1·8

The results show that there is slight oxidation of the vitamin in the presence of the dialysed lucerne juice indicating thereby the presence of a feeble ascorbic acid oxidase in lucerne. On the other hand the protection of vitamin C against oxidation by fresh undialysed lucerne juice indicates the presence of a powerful protective factor in lucerne.

Now with a view to study the nature of the protective factor, the effects of boiled, fresh and dialysed lucerne juice, respectively and that of the contents that were dialysed out on dialysing fresh lucerne juice, on pure vitamin C oxidation were determined. The results are summarized in Table III.

TABLE III

Influence of boiled, fresh and dialysed lucerne juice and the substances removed by dialysis on Vitamin C oxidation

	Mg. of Vitamin C in the total volume of the reaction mixture after incubation for			
	0 min.	30 min.	60 min.	120 min.
Vitamin C alone ..	5·0	4·0	3·2	2·4
Vitamin C + 2 c.c. of the (concentrated solution of the contents dialysed out)	5·0	4·8	4·8	4·8
Vitamin C + 2 c.c. of the boiled fresh lucerne juice	5·0	5·0	5·0	5·0
Vitamin C + 2 c.c. of the boiled dialysed lucerne juice	5·0	4·8	4·6	4·4

A concentrated solution of the substances removed by dialysis was prepared by boiling the water containing them to a very small volume. Dialysis of the juice was done as stated before. The reaction mixtures consisted of 10 c.c. M/5 acetate buffer (pH 5·6), 5 c.c. vitamin C solution

containing 5 mg. of the vitamin and 2 c.c. of the different solutions of the lucerne juice. The final volume in each case was made up to 20 c.c. The mixtures were incubated at 30° C. and at stated intervals of time, the vitamin C content in 2 c.c. aliquots of the mixtures was determined by the usual dyetitration method.

The results clearly point out that the protective factor in lucerne is quite thermostable and that it is distributed both in the dialysable and the undialysable portions of the juice.

Isolation of the enzymic and protective factors for Vitamin C oxidation from lucerne.—The method of Giri and Krishnamurthy (1940, 1941) was followed for the isolation of the protective factor, which has been reported to be widely distributed in plants (Giri and Krishnamurthy, 1940, 1941). 50 c.c. of fresh lucerne juice was centrifuged and the clear liquid was kept in the cold room overnight. It was then filtered and the clear filtered juice was treated with twice its volume of pure distilled acetone. The precipitate was quickly filtered off and washed with peroxide-free ether. The washed precipitate was dissolved in 50 c.c. water and the resulting solution was centrifuged to remove any insoluble matter. The clear supernatant liquid contained the enzyme which accelerates the oxidation of vitamin C. This solution was used for the enzymic oxidation of vitamin C in the subsequent experiments.

The acetone extract obtained after removal of the precipitate was concentrated on the water-bath to remove all the acetone and the resulting concentrated extract contained the protective factor. For convenience, the protective factor is termed as P.F. in the following experiments.

Influence of the P.F. on the non-cupric, cupric ion, and enzymic oxidation of Vitamin C.—The following reaction mixtures were set up containing:—

(1)	10 c.c. M/5 acetate buffer + 2 c.c. vitamin C (5 mg.)	+ 8 c.c. water (pH 5·6).
(2)	Do.	+ 4 c.c. P.F., 4 c.c. water.
(3)	Do.	+ 1 c.c. $CuSO_4 5H_2O$ solution (10·7 γ Cu), 7 c.c. Water.
(4)	Do.	+ 1 c.c. $CuSO_4$, $5\ H_2O$ solution (10·7 γ Cu), 4 c.c. P.F., 3 c.c. Water.
(5)	Do.	+ 4 c.c. enzyme solution, 4 c.c. Water.
(6)	Do.	+ 4 c.c. enzyme solution, 4 c.c. P.F.

The reaction mixtures were incubated at 30° C. and at stated intervals of time, the vitamin C was estimated in each by titrating 2 c.c. aliquots of the mixtures with 2:6 dichlorophenol indophenol. The results are summarized in Table IV.

TABLE IV

Effect of the protective factor in lucerne on the non-cupric, cupric ion and enzymic oxidation of Vitamin C

	Mg. of Vitamin C in the total volume of the reaction mixture after incubation for			
	0 min.	30 min.	60 min.	120 min.
Vitamin C alone ..	5·0	4·0	3·2	2·4
Vitamin C + P. F. ..	5·0	5·0	5·0	5·0
Vitamin C + Cu ..	5·0	3·0	2·0	1·2
Vitamin C + Cu + P. F. ..	5·0	5·0	4·9	4·8
Vitamin C + Enzyme ..	5·0	3·8	3·0	2·0
Vitamin C + Enzyme + P. F. ..	5·0	3·8	3·0	2·0

The results clearly show that the protective factor in lucerne annuls the non-cupric and cupric ion oxidation of vitamin C without having any effect on the enzymic oxidation of the vitamin.

The stability of vitamin C in lucerne on drying was then investigated.

Stability of vitamin C in lucerne on drying.—The lucerne leaves were dried by two different methods: (*a*) in direct sunlight and (*b*) in the laboratory under the fan at 25° C. The dried lucerne in each case was then powdered and the vitamin C content per gramme of the powder in each case was determined by titration with 2:6 dichlorophenol-indophenol. The results are summarized in Table V.

TABLE V

Effect of Drying on the Vitamin C content of lucerne

	Mg. of Vitamin C per gramme of the material
Fresh lucerne leaves ..	1·78
Lucerne leaves dried in direct sunlight ..	0·30
Lucerne leaves dried under the fan at 25° C..	0·28

The results definitely show that lucerne loses more than 80% of vitamin C on drying either in direct sunlight or under the fan at 25° C.

Summary

Lucerne (*alfa alfa*) is found to be a very rich source of vitamin C containing 1·78 mg. of the vitamin per gramme of the fresh lucerne as judged by the usual dye-titration method and Lugg's formaldehyde method.

Lucerne loses more than 80% of its vitamin C on drying either in direct sunlight or under the fan at 25° C.

Lucerne contains a feeble oxidase system and a powerful protective factor, which is very thermostable and which is associated partly with the colloidal and dialysable portions of the juice.

The protective factor is isolated free from the oxidase system and it is found to annul the non-cupric and cupric ion oxidation of vitamin C, without having any effect on the enzymic oxidation of the vitamin.

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CONTROL OF "RED ANTS" (*OECOPHYLLA SMARAGDINA* FAB.)

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Introductory

During the past several years, the problem of combating the common 'red ant' (*Oecophylla smaragdina* F.) on different fruit trees like *Mosumbi, Chickoo,* Mango and Guava has been receiving considerable attention. Sometime back, the senior author tried the utility of *banding the trees* with a mixture of Rosin and castor oil which yielded effective check against the migrating ants only. Recently, however, efficacy of DDT and BHC was worked out for the control of these insects, which although not serious pests, are a great nuisance while harvesting the crop. The results of our investigations are given below:

Material and Methods

The trials were conducted at the Ganeshkhind Fruit Experimental Station (Kirkee) and Modibag (Poona) during the years 1947 and 1948. Preliminary trials with DDT and BHC were made on about 25 nests on different trees under field conditions. Under each nest, a cloth catch-net was provided to estimate the mortality after every few hours. Gammexane, Hexyclan and DDT were tested in strengths ranging from 2 to 5%. To increase the residual effect of these insecticides a few deterrents like sulphur and naphthalene were also mixed. Field experiments were conducted in two series: (*a*) treating the nests only and (*b*) treating the entire trees along with the nests. Under each series, a further differentiation was made: (i) dusting after piercing the nests and (ii) without piercing them. The trials were replicated six to eight times with about 12 trees under each

treatment. The dusted trees and the nests were labelled to determine their reinfestation. Peerless dust guns were utilised for these trials and their nozzles were used in piercing and disturbing the nests.

Results

The data (Table I) shows relatively higher knockdown with 5% Gammexane (0·65% gamma isomer). After about 6 hours, however, its performance increased considerably up to a period of 24–48 hours. Of the other insecticides, Hexyclan 5% (0·65% gamma isomer) was relatively superior to DDT 5%.

Table I

Preliminary trials with different formulations of DDT and BHC on Oecophylla smaragdina *F. during* 1947–48

(Nests treated after piercing)

Insecticide	Formulation	Actual toxicant of BHC or DDT	Percentage mortality							
			1947				1948			
			1 hr.	6 hr.	24 hr.	48 hr.	1 hr.	6 hr.	24 hr.	48 hr.
DDT	Guesarol 403	3% DDT	0	52·5	80·1	85·0	0	50·0	70·0	80·0
	Guesarol 405	5% DDT	5·1	63·1	90·0	93·1	0	60·5	95·0	90·0
BHC	3% Gammexane	0·39% gamma isomer	15·0	55·8	70·0	80·0	5·0	55·0	85·0	90·8
	5% Gammexane (D. 025)	0·65% gamma	25·8	85·6	96·0	100·0	10·0	85·0	100·0	100·0
BHC	3% Hexyclan	0·39% gamma	5·1	50·1	60·0	90·0	5·0	50·1	70·0	85·0
	5% Hexyclan	0·65% gamma	10·8	80·3	90·5	95·0	10·0	60·8	95·0	99·0

For further trials, therefore, only 5% strengths of all these insecticides were taken (Table II) and applied with or without piercing the nests. When applied after piercing the nests, the results were very encouraging and the mortality ranged from 85·6–100% with Gammexane and 81·5–90·0% with Hexyclan. The relative performance was almost similar when sulphur or naphthalene was mixed with these insecticides. However, it was observed that treating the nests without piercing them is of little use.

TABLE II

Mortality of the adults and grubs of Oecophylla smaragdina *F. by treating nests only*

Insecticide	Method of application	Mean percentage mortality of grubs and adults after			Remarks
		6 hrs.	24 hrs.	48 hrs.	
Guesarol 405, DDT 5% ..	Nest pierced	60·0	80·9	95·3	On an average, the insecticides were applied at 1 oz. per nest
	Nest undisturbed	15·3	30·8	50·4	
Gammexane 5% D·025, 0·65% gamma	Pierced	85·6	98·4	100·0	
	Undisturbed	20·5	25·1	58·3	
Hexyclan 5%, 0·65% gamma ..	Pierced	81·5	93·5	99·0	
	Undisturbed	12·8	20·4	48·4	
Gammexane 5% plus sulphur (2:1)	Pierced	90·3	99·0	100·0	
	Undisturbed	30·4	41·6	63·8	
Hexyclan 5% plus sulphur (2:1)	Pierced	85·9	95·0	100·0	
	Undisturbed	21·3	31·6	50·2	
Guesarol 405 plus sulphur (2:1)	Pierced	78·0	89·0	98·4	
	Undisturbed	15·3	40·3	58·0	
Gammexane 5% plus naphthalene (8:1)	Pierced	74·0	95·8	99·7	
	Undisturbed	16·8	30·8	40·8	
Hexyclan 5% plus naphthalene (8:1)	Pierced	80·1	90·0	98·8	
	Undisturbed	13·0	21·8	45·7	
Guesarol 405 plus naphthalene (8:1)	Pierced	68·0	80·8	95·0	
	Undisturbed	12·0	18·8	39·8	
Untreated (check)	Pierced	2·0	3·5	4·0	
	Undisturbed	0·0	0·0	0·0	

The data in Table III however, shows that with the addition of sulphur to the insecticides the trees remained uninfested even after 12 weeks when the nests were dusted after piercing.

ECONOMICS OF THE TREATMENTS

In order to get satisfactory control of these insects the trees should be dusted entire after piercing the nests. The quantity of dust required per tree of 10–15 feet size was about 1 lb. and two labourers could finish 30 trees per day with one dust gun. The details of the economics are given in Table IV.

It is obvious from the above data that, Hexyclan was the cheapest of all insecticides, Gammexane being the next. With the addition of sulphur however, 5% Hexyclan treatment became still cheaper and the insecticides in order of their merit were Hexyclan 5%, Gammexane 5% and DDT 5%. However, seeing the relative performances of all these insecticides alone or

TABLE III

Mortality of the adults and grubs of Oecophylla smaragdina *F. by dusting entire trees along with the nests*

Insecticide	Method of application	Average percentage mortality of grubs and adults after			Percentage of nests and trees reinfested after						Remarks
					4 weeks		8 weeks		12 weeks		
		6 hrs.	24 hrs.	48 hrs.	Nests	Trees	Nests	Trees	Nests	Trees	
Guesarol 405 ..	(i) Nests pierced	64·0	91·9	98·3	0·0	2·0	0·0	4·0	0·0	7·0	On an average, insecticides were applied at the rate of 1 lb. per tree
	(ii) Undisturbed	20·8	30·1	48·0			All infested				
Gammexane 5% ..	(i) Pierced	80·6	98·0	100·0	0·0	3·0	0·0	5·0	0·0	8·0	
D·025 ..	(ii) Undisturbed	25·8	40·8	51·3			All-infested				
Hexyclan 5% ..	(i) Pierced	80·0	93·0	98·9	0·0	3·4	0·0	6·2	0·0	8·4	
	(ii) Undisturbed	12·0	18·4	48·3			All infested				
Gammexane 5% ..	(i) Pierced	90·0	95·0	100·0	0·0	0·0	0·0	0·0	0·0	0·0	
Plus sulphur (2:1) ..	(ii) Undisturbed	25·4	48·7	60·7			All infested				
Hexyclan 5% ..	(i) Pierced	83·7	90·8	100·0	0·0	0·0	0·0	0·0	0·0	0·0	
Plus sulphur (2:1) ..	(ii) Undisturbed	20·8	32·5	53·0			All infested				
Guesarol 405 ..	(i) Pierced	70·7	90·0	99·0	0·0	0·0	0·0	0·0	0·0	0·0	
Plus sulphur (2:1) ..	(ii) Undisturbed	20·4	38·7	48·0			All infested				
Gammexane 5%+ ..	(i) Pierced	88·5	94·7	98·0	0·0	3·4	0·0	5·6	5·6	8·9	
Naphthalane (8:1) ..	(ii) Undisturbed	18·6	31·8	40·8			All infested				
Hexyclan 5%+ ..	(i) Pierced	80·7	90·8	97·9	0·0	2·0	0·0	6·3	6·3	8·8	
Naphthalene (8:1)	(ii) Undisturbed	18·0	25·6	41·3			All infested				
Guesarol 405 + ..	(i) Pierced	69·8	89·0	95·8	0·0	3·0	0·0	4·8	4·8	6·9	
Naphthalene (8:1)	(ii) Undisturbed	15·6	32·5	30·8			All infested				
Untreated ..	(i) Pierced	2·0	2·8	5·0	100·0	100·0	100·0	100·0	100·0	100·0	
	(ii) Undisturbed	0·0	0·0	0·0	100·0	100·0	100·0	100·0	100·0	100·0	

TABLE IV

Economics of insecticidal control of 'red ants' (Oecophylla smaradgina *F.*)

Treatment	Cost of insecticide per tree	Labour per day	Cost of Labour per tree	Sundry charges per tree	Total cost of treating one tree
	Rs. A. P.	Rs. A. P.	Rs. A. P.	Rs. A. P.	Rs. A. P.
1 Guesarol 405 ..	0 12 0	1 8 0	0 1 6	0 0 6	0 14 0
2 Gammexane 5% ..	0 9 0	1 8 0	0 1 6	0 0 6	0 11 0
3 Hexyclan 5%	0 5 0	1 8 0	0 1 6	0 0 6	0 7 0
4 Gammexane 5% + sulphur (2:1)	0 7 0	1 8 0	0 1 6	0 0 6	0 9 0
5 Hexyclan 5% + sulphur (2:1)	0 4 0	1 8 0	0 1 6	0 0 6	0 6 0
6 Guesarol 405 + sulphur (2:1)	0 9 0	1 8 0	0 1 6	0 0 6	0 11 0
7 Gammexane 5% + naphthalene (8:1)	0 12 0	1 8 0	0 1 6	0 0 6	0 14 0
8 Hexyclan 5% + naphthalene (8:1)	0 8 6	1 8 0	0 1 6	0 0 6	0 10 6
9 Guesarol 405 + naphthalene (8:1)	0 15 6	1 8 0	0 1 6	0 0 6	1 1 6

in combination with sulphur and considering their economics, Gammexane 5% and Hexyclan 5% are recommended.

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ACTIVE ELONGATION OF UNSTRIATED MUSCLE DURING INHIBITION, ACCOMMODATION AND RELAXATION

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Ramsey and Street (1941) have shown that a single frog muscle fibre floating in Ringer's solution returns spontaneously to its resting length after stimulation provided that it is not allowed to shorten more than one third of its length. This relaxation is active; it is more rapid than can be accounted for by any external force acting upon the fibre. Hill (1949) found no evidence of lengthening during relaxation in an unloaded muscle except probably by elastic forces at lengths below 60 p.c. Singh (1944 *a*) and Singh and Singh (1946 *a*) described active elongation of frog's stomach muscle; this will be further described in the next paper. Singh, Singh and Muthana (1947) found that fowl's gut muscle elongated when stimulated with alternating current in sodium deficient solutions.

Satisfactory evidence of active elongation of dog's stomach muscle has been obtained recently (Singh and Singh, 1948 *b*). In the present research, these experiments have been extended and the effect of various substances on active elongation and its relation to tonus described. Active elongation of the human appendix has also been investigated.

Experimental

Most experiments were performed on pieces of dog's stomach muscle. A few were performed on the human appendix. Active elongation was studied by laying the muscle in a trough and stimulating it with electric current or chemicals. The trough was emptied of saline and the two electrodes were lightly placed on the muscle. The length was measured and the current passed. When the maximum shortening had occurred, the length was again measured and the electrodes removed. The length was subsequently measured at intervals till a constant value was reached.

For stimulation by chemicals the muscle was laid as above in a trough and the solution containing the chemical poured into the chamber. When stimulation was to be stopped, the chemical was replaced with the saline. With certain stimulants, the dog's stomach muscle contracts so rigidly, that it can be gently lifted and placed from the trough containing the stimulant into another containing saline without elongation occurring, the elongation then begins after the muscle has been placed in the saline.

The saline used was as described previously (Singh, 1940), but was buffered with borate, the pH being 8·1. The term " potassium saline ", etc., indicates that the sodium of the saline has been replaced with potassium, etc.

After removal from the animal, the pieces of muscle were aerated for about half an hour in saline so that they attained a constant length. These experiments were performed at about 25° C. Below 20° C. and above 30° C., the elongation is not good as the tone greatly increases. Under these conditions it is possible to obtain consistent results for about 3–4 hours, the muscle being stimulated once in 15–30 minutes. The muscle was immersed in various experimental solutions for 30 minutes. The various calculations involved in each experiment are given in Table VIII. Thus if the initial length of the muscle is x and during contraction it is y, and after relaxation it is z, then the percentage of relaxation is given by the formula $\frac{100\,(z-y)}{(x-y)}$.

Results

Dog's stomach muscle relaxes actively after being stimulated with electric current (Singh and Singh, 1948 *b*), vagus nerve and chemicals, the various ions replacing the sodium or chloride of the saline. The chemicals tried have been monovalent cations, potassium, ammonium, sodium (sodium chloride of the saline being replaced with sucrose, which caused contraction), monovalent anions, bromide, nitrate, iodide, thiocyanate, divalent cations, strontium, barium, magnesium and drugs acetylcholine, pilocarpine, nicotine and histamine. Withdrawal contractions may prevent relaxation after ammonium, nitrate and thiocyanate (Singh, 1939, 1940). Thus in 7 experiments, when stimulated through the vagus nerve, the muscles contracted to 89, 73, 85, 86, 95, 92 and 94 p.c. of the initial length respectively, and relaxed actively to 98, 93, 97, 94, 100, 102 and 104 p.c. respectively. In 6 experiments, when stimulated with potassium saline, the muscles contracted to 63, 68, 73, 80, 88 and 73 p.c. and relaxed actively to 68, 90, 93, 80, 104 and 81 p.c. respectively. In 6 experiments, when stimulated with ammonium saline, the muscles contracted to 93, 86, 92, 110, 90 and 107 p.c. and relaxed to 113, 83, 92, 109, 91 and 93 p.c. respectively, some muscles having initially elongated rather than contracted. In 6 experiments, when stimulated with barium saline, the muscles contracted to 54, 66, 64, 60, 49 and 55 p.c. respectively and relaxed actively to 54, 80, 74, 69, 49 and 70 p.c. respectively. In 6 experiments, when stimulated with acetylcholine (1 in 10,000), the muscles contracted to 91, 88, 92, 88, 88 and 89 p.c. respectively and relaxed actively to 91, 107, 100, 100, 100 and 100 p.c. respectively. In 6 experiments, when stimulated with histamine (1 in 10,000), the muscles contracted to 90, 90, 93, 84, 94 and 90 p.c. respectively and relaxed actively to 100, 101, 100, 100, 94 and 90 p.c. respectively.

The muscle also relaxes actively after spontaneous or rhythmic contractions. Rhythmic contractions may occur just after removal from the animal or after previous treatment with barium or other stimulants. Rhythmic contractions are only possible if the muscle relaxes each time, so that such contractions of any unloaded muscle implies active relaxation.

Frog's skeletal muscle is known to contract rhythmically in calcium-free alkaline solutions; cardiac cells in tissue cultures are known to beat rhythmically. Therefore relaxation in skeletal and cardiac muscles is active.

The human appendix also relaxes actively. Thus in 6 experiments, when stimulated with alternating current (12 volts for 10 seconds), the muscle contracted to 90, 90, 93, 84, 94 and 90 p.c. and relaxed actively to 100, 101, 100, 100, 94 and 90 p.c. respectively. In the last two experiments, the appendix was tonically contracted and the relaxation absent.

Active relaxation during inhibition.—When the dog's stomach muscle is stimulated with potassium, there may be preliminary inhibition during which it elongates actively (Fig. 1). Thus in 12 experiments, when stimulated with

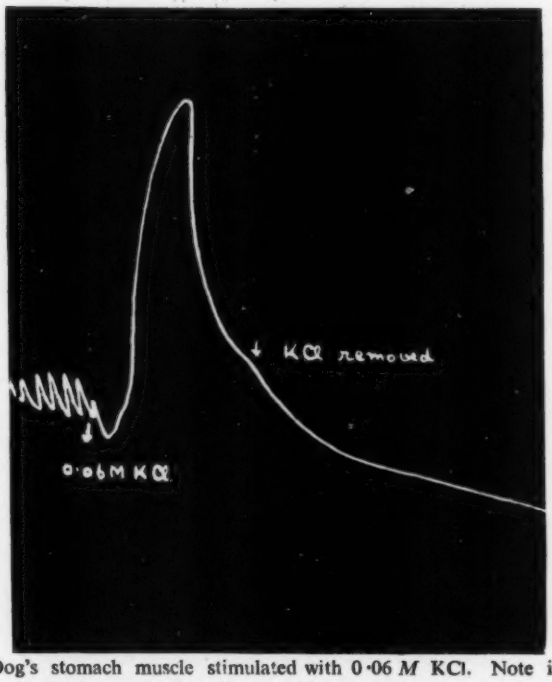


FIG. 1. Dog's stomach muscle stimulated with 0·06 *M* KCl. Note initial relaxation and then the tension begins to decline after reaching peak though potassium is still present. On relaxing, the tension falls below the original line.

potassium saline, 7 muscles elongated to 112, 110, 107, 102, 112, 110 and 121 p.c. of their initial length respectively, the rest having contracted. Ammonium, as mentioned above and thiocyanate also produce similar results (Singh, 1940). Thus in 6 experiments, when stimulated with thiocyanate saline, 4 muscles elongated to 105, 113, 111 and 111 p.c. of their initial length respectively, the rest having contracted. Inhibition, therefore, may be active. This preliminary inhibition in unstriated muscle resembles the latency relaxation in striated muscle (Sandow, 1945; Singh, 1946), and is abolished by asphyxia, cyanide and iodoacetic acid.

Inhibition may also be passive, as shown by the fact that adrenaline produces no elongation though it may do so if the muscle is loaded. These results are in accord with the oxygen consumption experiments (Rao and Singh, 1940) and with the effect of glucose and oxygen (Singh, 1949; Singh and Singh, 1948 *a*, *c*). Thus during inhibition by ammonium and sodium, the oxygen consumption increases; asphyxia abolishes the inhibition which is restored with glucose. During inhibition by adrenaline, the oxygen consumption decreases. The preliminary inhibition precludes elongation by elastic forces, as has been suggested by Hill (1949) in skeletal muscle.

Active relaxation during accommodation.—If stimulation such as by alternating current, potassium acetylcholine vagus is continued, then the muscle relaxes due to accommodation (Singh, 1938 *b*). During such accommodation relaxation is active. Accommodation is decreased by cyanide (Singh, 1938 *b*) and asphyxia (Singh, 1949). Active relaxation during accommodation is well shown if the muscles are stimulated with nicotine and pilocarpine. Thus in 6 experiments, when stimulated with nicotine (1 in 10,000), 6 muscles contracted to 70, 75, 59, 68, 64 and 65 p.c. respectively and relaxed to 100, 97, 105, 95, 95 and 108 p.c. of the initial length respectively, though the drug was still present in the saline. Subsequent transfer to saline produced no change. In another 6 experiments, when stimulated with pilocarpine (1 in 10,000), the muscles contracted to 80, 82, 82, 92, 73 and 89 p.c. and relaxed during accommodation to 100, 101, 98, 94, 118 and 101 p.c. of the initial length respectively. The muscle relaxes completely though the drug is present in the solution. The muscle may relax beyond the initial length thus neutralising part of the tone. Inhibition and accommodation are identical (Singh, 1945). The relaxation beyond the initial length during accommodation precludes elastic forces as being the cause of relaxation. The muscle may recontract if kept long in chemicals, such as potassium, ammonium, barium, owing to the fact that tone producing substances antagonise the factor of accommodation (Singh, 1944 *b*, Fig. 2).

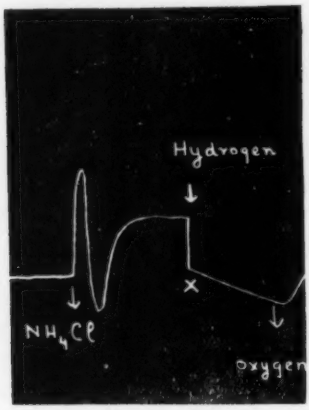


FIG. 2. Dog's stomach muscle, stimulated with ammonium saline throughout. Drum stopped at X for 10 minutes.

If the muscle contracts, active relaxation during accommodation is diminished or abolished by asphyxia cyanide and iodoacetic acid.

After ordinary contraction, relaxation may proceed beyond the initial length. This resembles the increase of positive potential which follows the spike and negative potential in nerve. With dog's stomach muscle, it is well shown if stimulated with alternating current, potassium and acetylcholine. Relation between accommodation and relaxation is shown by the fact that substances that diminish accommodation also decrease relaxation. Thus in *Mytilus* muscle both accommodation as well as relaxation are decreased in the absence of calcium (Singh, 1938 *b*, *c*). Cyanide, barium and other tone producing substances produce the same effect. In dog's stomach muscle also, active relaxation and accommodation are interfered with by tone producing substances. This shows that inhibition, accommodation and active relaxation are closely related.

Relation between contraction and relaxation.—Out of 216 experiments, in only one did the muscle contract between 40–50 p.c. of the initial length. In 6 experiments it contracted to 50–60 p.c. In about 85 p.c. of the experiments, the muscles contracted between 60 to 80 p.c. There was no relation between the degree of contraction and relaxation. A small contraction may not be followed by relaxation.

Sometimes atonic stomachs were found in animals. Pieces of muscle from these stomachs (3 experiments), did not contract when aerated in saline, but contracted when stimulated with electric current. From initial length of 180, 185, 183 mm., these muscles contracted to 36, 38 and 35 p.c. of these

lengths respectively and did not relax beyond 44 p.c. Thereafter they contracted and relaxed as usual but not beyond 44–45 p.c. This is akin to the delta state in striated muscle fibres (Ramsey and Street).

These muscles were then suspended in a muscle chamber, using isometric lever to keep the length constant, and their reactions resembled exactly those of normal muscles, so that normal tone appears to be due to failure of relaxation. Using isotonic lever, their stretch curves resembled those of normal muscles, there being no rapid stretching to their original length, though slowly, they stretched beyond that length. Such a locking has been described in previous papers (Singh, 1938 *a*, 1944 *b*), when stretch and release curves were recorded. Thus the muscle may not contract when kept extended, but when allowed to shorten for sometime it contracts; it will then stretch very slowly to its original length. The asymmetrical nature of the stretch and release curves suggests that the nature of locking is electrical. In frog's stomach, this locking can be abolished by distilled water which causes active elongation. The locking is, therefore, due to contraction.

There is no relation between the locking and extent of contraction. Thus locking may occur in dog's stomach muscle in saline, even if it is allowed to shorten by one per cent. after being previously stretched, and the time required may be just necessary to lift the lever by the finger. Thus normal tone is due to some kind of locking of the contractile proteins. By this means the hollow viscera adapt themselves to their contents without alteration of tension. The processes of contraction and relaxation are distinct, as shown by the fact that they may be affected oppositely. Thus in *Mytilus* muscle, cyanide, and barium may increase contraction, but diminish relaxation.

The curve of active relaxation.—This is shown in Fig. 3. Relaxation is usually complete within a minute, though it may take 2–3 minutes. Relaxation is usually brisk just after removal from the animal; soaking it in saline causes it to become slower.

Effect of frequent stimulation.—As the muscle is stimulated frequently once a minute, it may relax more during the first two or three contractions, and thereaftre it relaxes less and less till it may fail to relax, or the muscle may fatigue from the outset.

Effect of strength of stimulus.—If the stimulus is very strong (25–50 volts), then contraction is powerful, up to 30–40 p.c. of the initial length, and the relaxation may be absent. This again resembles the delta state of striated muscle fibres. If the unrelaxed muscle is suspended in a muscle chamber, its reactions are like those of a normal muscle, though the

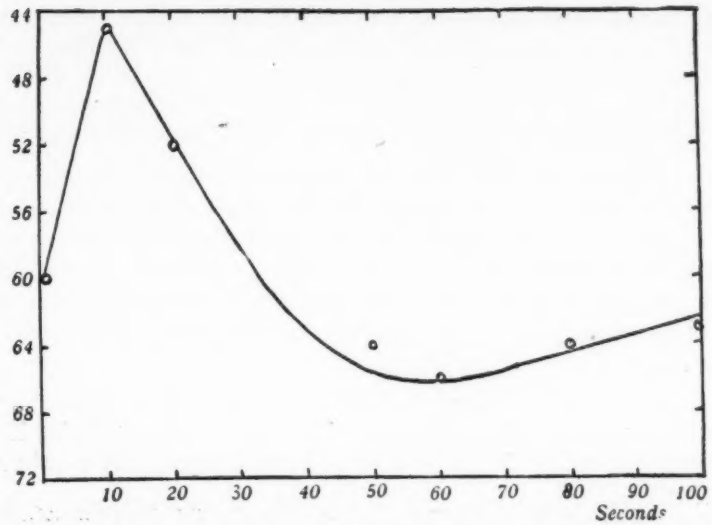


FIG. 3. Dog's stomach muscle stimulated with alternating current; shows curve of relaxation.

excitability is diminished. It shows tone, which thus appears to be due to failure of relaxation.

Effect of asphyxia.—If the muscle is stimulated with alternating current, asphyxia has no significant effect on relaxation, so that energy for relaxation is anaerobic (Table I). Relaxation is diminished only in experiments

TABLE I. *Dog's stomach muscle. Effect of asphyxia on active relaxation o contraction produced by stimulating with alternating current*

Experimental	Relaxation p.c.						
	No. of experiment						Average
	1	2	3	4	5	6	
Oxygen ..	71	71	63	60	52	45	60
Asphyxia ..	65	60	70	50	52	65	60
Asphyxia and 0·1 p.c. glucose	44	68	58	66	77	77	65
Oxygen and glucose	63	60	44	78	66	54	61

Each of the experimental procedures involved 30 minutes.

1, 2 and 4. In others it is either increased or unaffected. The increase in relaxation is akin to increase of contraction during asphyxia. Glucose 0·1 p.c., improved relaxation in 4 and diminished in 2 muscles, the diminution is akin to the similar action of glucose on contraction. Further oxygenation diminished relaxation in 4 muscles, as may happen with contraction.

These experiments show that the anaerobic metabolic mechanism for relaxation is antagonistic to the aerobic one and the glycolytic and non-glycolytic anaerobic mechanisms are mutually antagonistic; glucose is utilised differently under aerobic and anaaerobic conditions, as oxygen has inhibitory action on asphyxiated muscle soaked in glucose. These effects on relaxation resemble those on contraction (Singh, 1949; Singh and Singh, 1949 *a*, *b*, *c*, *d*).

If the muscle is stimulated with potassium, relaxation does not occur at all in the absence of oxygen even if glucose is present (Table II). If asphyxiated, the muscle further contracts due to asphyxial hyperexcitability.

TABLE II. *Dog's stomach muscle. Effect of asphyxia on contraction and relaxation by potassium saline*

Initial length = 100 p.c.

No. of Experiment	Length during contraction p.c.	Length during asphyxia p.c.	Length during asphyxia with 0·1 p.c. glucose p.c.	Relaxation p.c.
1	57	54	54	0
2	84	81	81	0
3	76	74	74	0
4	86	85	85	0
5	71	63	63	0
6	78	70	70	0

The muscle was immersed in potassium saline, then asphyxiated for 30 minutes, and then glucose was added to the potassium saline and length recorded after 30 minutes.

If the muscle is loaded then the relaxation is rapid. This conclusively proves the existence of two kinds of relaxation, one active and the other passive. These experiments show the difference between the relaxation of tonic and twitch contractions.

Cyanide produces effects similar to those of asphyxia, glucose producing partial relief (Tables III and IV).

TABLE III. *Dog's stomach muscle stimulated with alternating current. Effect of cyanide on active relaxation.*

.. mean that the muscle has become inexcitable.

Concentration of NaCN	Relaxation p.c.						
	No. of experiment						Average
	1	2	3	4	5	6	
0 ..	78	56	66	73	80	74	71
1 in 10^5 ..	64	76	33	55	66	47	57
1 in 10^4 ..	0	0	..	..	..	0	0
1 in 10^4 with 0·1 p.c. glucose	20	48	75	50	100	46	57

TABLE IV. *Dog's stomach muscle stimulated with potassium saline. Effect of sodium cyanide* (1 *in* 10^4) *on contraction and active relaxation*

Initial length = 100 p.c.

No. of Experiment	Length during contraction	Length in NaCN and potassium	Length in NaCN, 0·1 p.c. glucose and potassium	Relaxation due to glucose p.c.
1	88	86	80	0
2	96	92	96	50
3	88	92	105	163
4	87	87	96	69
5	84	83	88	30
6	96	93	96	43

Effect of iodoacetic acid.--Iodoacetic acid (1 in 20,000) diminishes relaxation (Table V). In asphyxiated muscles, relaxation is abolished provided they contract. The effect of iodoacetic acid shows that the sources of energy for relaxation and contraction are similar. Lactic acid is therefore not necessary for relaxation. Energy must therefore be provided at first instance by the breakdown of creatine phosphate and adenosine triphosphate.

TABLE V. *Dog's stomach muscle stimulated with alternating current. Effect of iodoacetic acid,* 1 *in* 20,000 (*I.A.A.*) *on active relaxation*

.. mean that the muscle has become inexcitable.

Experimental	Relaxation p.c.						
	No. of experiment						Average
	1	2	3	4	5	6	
Normal ..	66	87	80	74	80	75	77
1. A. A. ..	43	60	82	65	77	60	65
1. A. A. and Asphyxia ..	0	..	0	0	..	..	0
1. A. A. and Oxygen ..	0	0	0	..	0	..	0

With potassium the relaxation is completely prevented in muscles poisoned with iodoacetic acid (6 experiments). This again shows the difference between the relaxation of tonic and twitch contractions.

Effect of temperature.—In 6 experiments (Table VI) the muscles were at first stimulated at 30–31° C. The temperature was then lowered to

TABLE VI. *Dog's stomach muscle stimulated with alternating current. Effect of temperature on active relaxation.*

.. mean that the muscle has become inexcitable.

Temperature 0° C.	Relaxation p.c.						
	No. of experiment						Average
	1	2	3	4	5	6	
31	45	52	45	64	79	71	59
11	0	0	..	..	..	..	0
20	56	48	83	33	54	32	51
25	35	33	75	50	90	80	62
30	61	85	43	33	22	140	64
35	0	..	..	..	0	0	0
30	0	0	..	..	..	10	2

10–11° C. and readings taken. It was then gradually increased to 35° C. and readings taken at 15, 20, 25, 30 and 35° C. The temperature was again lowered to 30, 25° C. Below 20 and above 30° C. relaxation is impaired and absent at 15 and 35° C., the optimum temperature being the same as that for contraction, that is, about 30° C. If the temperature is reduced after being raised to 35° C. relaxation is never as good as before, being permanently impaired. An interesting point is that just after removal from the animal, relaxation is very brisk at body temperature, so that the saline in some way impairs the property of the muscle to contract as well as to relax. This difference between the properties of the muscle in the body and in saline respectively, is being investigated.

Changes in length of unloaded unstriated muscle also take place with variations of temperature (Table VII). If the muscle is cooled from 30 to 4° C., then it contracts and partially relaxes again with increase of temperature. The cause of contraction with lowering of temperature follows

TABLE VII. *Dog's stomach muscle. The effect of temperature on length (p.c.) of unstimulated and unloaded muscle.*

Initial length = 100 p.c.

Temperature 0° C.	No. of experiment						Average
	1	2	3	4	5	6	
31	100	100	100	100	100	100	100
4	80	97	89	98	82	71	86
10	85	97	89	97	74	75	86
20	85	97	89	98	75	75	87
30	89	99	92	98	82	75	89
36	80	74	85	68	60	65	72
28	80	75	65	64	60	65	68

from the fact that energy is required to keep the muscle elongated (Singh, 1949; Singh and Singh, 1948 *c*). This view is supported by the further observation that the muscle contracts if the temperature is raised to 35° C., but does not elongate actively if the temperature is again lowered, though passive relaxation occurs. Again if heated to 50° C., it dies, but does not actively elongate, though it relaxes readily if stretched.

Effect of direct current.—When stimulated with direct current, active relaxation is retarded. Thus in 6 experiments, the relaxation was 100, 100,

120, 100, 120 and 100 p.c. repectively when the muscle was stimulated with alternating current. When stimulated with direct current (14 volts for 10 seconds), the relaxation was 22, 100, 50, 75, 80 and 0 p.c., the last muscle not relaxing at all. Alternating current neutralises the effect of direct current. This is probably due to the fact that when the muscle is stimulated a large amount of energy is liberated, as the oxygen consumption is doubled (Rao and Singh, 1940).

Effect of monovalent cations

Effect of potassium.—Potassium is an important cation, as it produces a tonic contraction and the relaxation between tone and relaxation can be well shown. Replacement of 5 p.c. of the sodium of the saline usually abolishes relaxation (Table VIII), though 10–15 p.c. replacement may be required. If 20 p.c. of the sodium of the saline is replaced, then the muscle becomes inexcitable. After immersion in saline containing substances causing tonic contraction, recovery is never complete, as the muscle becomes more sensitive and the tone increases, which diminishes relaxation.

Potassium also diminishes relaxation of contraction produced by acetylcholine. Thus in 6 experiments, the relaxation was 133, 100, 300, 140, 100 and 100 p.c. respectively when the muscle was stimulated with acetylcholine (1 in 10,000). After treatment for half an hour with saline in which 5 p.c. of the sodium had been replaced with potassium, the relaxation was 25, 0, 0, 50, 0 and 0 p.c. respectively. If immersion is prolonged, then potassium antagonises relaxation of contraction produced by itself. Thus if the time of immersion be one hour, then relaxation is practically absent. Thus in 6 experiments, the relaxation was 75, 61, 57, 29, 42 and 62 p.c. respectively when the muscle was stimulated with potassium saline. After immersion for one hour in the potassium saline, the relaxation was 0, 0, 0, 0, 14 and 0 p.c. respectively.

In the absence of potassium, relaxation is either diminished or increased. Thus in 6 muscles, relaxation in the presence of potassium was 70, 100, 81, 50, 75 and 62 p.c. respectively. In the absence of potassium it was 50, 100, 71, 50, 66 and 76 p.c. respectively. These differences are probably related to tone. It is probable that the effect of potassium deficiency is to decrease relaxation, but an opposite effect may be produced by diminution of tone. On reintroduction of potassium the relaxation was 70, 60, 72, 66, 50 and 30 p.c. respectively, diminishing in 3 muscles. This diminution is probably due to increase of tone, as the muscle becomes more sensitive to potassium after being deprived of it, so that the normal concentration of potassium acts like larger concentrations mentioned above.

TABLE VIII. *Dog's stomach stimulated with alternating current. Effect of potassium on active relaxation. 5 p.c. of the sodium of the saline replaced with potassium*

No. of experiment			Initial length	Length during contraction		Length during Relaxation		Relaxation
			mm.	mm.	p.c.	mm.	p.c.	p.c.
1	Saline		40	30	75	38	95	80
2			55	38	69	48	87	66
3			76	49	65	73	96	90
4			28	22	79	27	96	83
5			46	28	61	45	98	94
6			61	42	69	53	87	58
7			50	30	60	50	100	100
8			80	70	88	78	98	80
9			74	48	65	60	81	46
10			64	50	78	60	94	71
11			55	39	71	50	91	69
12			51	34	67	45	88	65
13			66	54	82	62	94	67
14			36	23	64	36	100	100
		Average			71		93	76
1	KCl		40	34	85	35	88	17
2			50	44	88	44	88	0
3			66	54	82	54	82	0
4			30	24	80	26	87	33
5			48	43	90	44	92	20
6			50	45	90	47	94	40
7			44	38	86	41	93	50
8			74	69	93	69	93	0
9			53	48	91	48	91	0
10			47	42	89	42	89	0
11			46	40	87	40	87	0
12			35	31	89	31	89	0
13			55	51	93	51	93	0
14			34	27	79	30	88	43
		Average			87		90	15
1	Saline		..	..	..	..	..	..
2			..	..	..	..	..	..
3			62	54	87	54	87	0
4			28	26	93	27	96	50
5			45	35	78	39	87	40
6			49	41	84	46	94	63
7			44	35	80	41	93	67
8			70	66	94	69	99	75
9			52	49	94	51	98	67
10			41	38	93	40	98	67
11			43	35	81	42	98	87
12			35	31	89	32	91	25
13			51	47	92	49	96	50
14			32	30	94	31	97	50
		Average			88		95	53

Effect of ammonium.—Ammonium diminishes relaxation. Thus in 6 muscles, relaxation in saline was 100, 73, 41, 58, 76 and 100 p.c. respectively. After replacement of 20 p.c. of the sodium of the saline with ammonium, the relaxation in these muscles was 75, 0, 50, 10, 16 and 9 p.c. respectively. On reintroduction into saline the relaxation was 75, 20, 0, 25, 43 and 66 p.c. respectively. On withdrawal of ammonium, tone may increase thus diminishing relaxation, as has happened in the third experiment.

Effect of lithium.—Lithium decreases relaxation, as it causes slight tonic contraction. Thus in 6 muscles, relaxation in saline was 75, 44, 83, 73, 50 and 80 p.c. respectively. In lithium saline, the relaxation was 45, 40, 50, 0, 33 and 50 p.c. respectively; again in saline the relaxation was 100, 36, 100, 50, 66 and 50 p.c. respectively.

Effect of sodium chloride.—Sodium chloride was replaced with sucrose. If sodium chloride of the saline is decreased, relaxation decreases, but it increases again in sodium chloride content, 20 p.c. of original. Thereafter relaxation is abolished. Thus in 6 muscles, relaxation in saline was 85, 88, 77, 88, 70 and 73 p.c. respectively. In saline in which 25 p.c. of the sodium chloride was replaced with sucrose, the relaxation was 62, 65, 90, 70, 88 and 42 p.c.; after 50 p.c. replacement of sodium chloride, the relaxation was 45, 25, 66, 65, 75 and 29 p.c. respectively. After 75 p.c. replacement, the relaxation was 83, 83, 100, 83, 83 and 100 p.c. respectively. In the absence of sodium chloride, relaxation was absent in all the muscles. When restored to saline, the relaxation was 80, 66, 50, 62, 75 and 66 p.c. respectively. These effects on relaxation resemble those on contraction (Singh, 1939). These effects are probably due to opposite actions of sodium and chloride (Singh, 1942), removal of sodium decreasing and removal of chloride increasing the relaxation. The net result depends upon as to which of these effects predominate.

Removal of 5–10 p.c. of the sodium chloride of the saline increases relaxation. This action may be produced up to 25 p.c. replacement of sodium chloride. These effects of sodium chloride on relaxation resemble those on contraction.

Effects of hydrogen ions.—The optimum pH for relaxation as well as contraction is about 8. Thus in 6 muscles, relaxation at pH 8·5 was 78, 73, 82, 85, 77 and 75 p.c. respectively. At pH 8, it was 78, 73, 82, 85, 77 and 75 p.c. respectively. At pH 7 it was 60, 66, 78, 77, 75 and 62 p.c. respectively. At pH 6·5, it was 50, 61, 73, 40, 58 and 35 p.c. respectively. At pH 6 it was 100, 60, 84, 56, 64 and 41 p.c. respectively. At pH 5·2, it was 0, 0, 0, 33, 20 and 0 p.c. respectively. When restored to pH 8, the relaxa-

tion was 0, 60, 0, 0, 23 and 37 p.c. respectively. As with contraction, relaxation again increases at about pH 6·5–6·0. This is probably due to the release of the metabolic mechanism for acid solutions (Singh and Singh, 1948 *a*; 1949 *a, b, c, d*). At pH 5·2, tonic contraction is produced, and relaxation is abolished.

A loaded unstriated muscle relaxes markedly if the reaction is changed from pH 8 to 6·5. An unloaded muscle however, shows little or no active elongation under such conditions. Thus in 6 experiments with unstriated and unloaded muscle, the lengths in pH 8·1 were respectively 47, 76, 79, 56, 48 and 47 mm. In pH 6, the lengths respectively were 45, 77, 81, 59, 46 and 45 mm. Reimmersion in pH 8·1 caused them to contract to 40, 68, 76, 54, 41 and 42 mm. respectively. This is another example of passive relaxation.

Effect of divalent cations

Effect of calcium.—Excess of calcium decreases relaxation. Thus in 6 muscles, relaxation in saline was 90, 74, 70, 81, 200 and 45 p.c. respectively. In saline in which 20 p.c. of the sodium chloride was replaced with calcium chloride, the relaxation was 65, 27, 90, 65, 62 and 67 p.c. respectively. In 40 p.c. calcium chloride, the relaxation was 25, 91, 70, 44, 44 and 36 p.c. respectively. In saline in which all the sodium chloride was replaced with calcium chloride, relaxation was 0, 18, 50, 50, 43 and 60 p.c. respectively. On restoration to saline, the saline was 44, 57, 83, 50, 33 and 63 p.c. respectively. In some muscles, relaxation again increases with increase in the concentration of calcium. This shows that calcium has two opposing actions. Its tone producing properties decreases relaxation, otherwise relaxation appears to be favoured by excess of calcium. The action of calcium thus appears to be identical in inhibition, accommodation and relaxation. In the absence of calcium, there are similarly two opposing actions. Thus in 6 muscles, in the presence of calcium, the relaxation was 80, 78, 74, 76 65 and 85 p.c. respectively. In the absence of calcium, the relaxation was 86, 75, 54, 76, 68 and 63 p.c. respectively. Again in the presence of calcium, the relaxation was 70, 100, 81, 50, 75 and 62 p.c. respectively.

Effect of strontium.—Strontium diminishes relaxation. Thus in 6 muscles, in saline the relaxation was 70, 77, 56, 61, 66 and 53 p.c. respectively. In saline, in which 50 p.c. of the sodium chloride was replaced with strontium chloride, the relaxation was 85, 70, 33, 40, 67 and 38 p.c. respectively. In saline, in which all the sodium chloride was replaced with strontium chloride, the relaxation was 66, 50, 0, 20, 0 and 50 p.c. respectively. On restoration to saline, the relaxation was 66, 75, 33, 42, 40 and 40 p.c. respectively.

Effect of barium.—Small concentrations of barium favour relaxation, and larger concentrations abolish it. Thus in 6 muscles, in saline the relaxation was 86, 75, 57, 55, 76 and 92 p.c. respectively, In saline in which 10 p.c. of the sodium chloride was replaced with barium chloride, the relaxation was 77, 93, 75, 87, 73 and 25 p.c. respectively. In saline with 20 p.c. sodium chloride replaced, the relaxation was 0, 0, 40, 0, 0 and 0 p.c. respectively. If immersed for one hour, like potassium, barium antagonises its own relaxation. Thus in 6 muscles, after stimulation with barium saline the relaxation was 84, 40, 28, 0, 0 and 32 p.c. respectively. After immersion for one hour in barium saline, the relaxation was absent in all the muscles.

Effect of magnesium.—Magnesium, like calcium, both increases and decreases relaxation in small concentrations. Thus in 6 muscles, relaxation in saline was 76, 75, 100, 65, 74 and 68 p.c. respectively. In saline in which 20 p.c. of the sodium chloride was replaced with magnesium chloride, the relaxation was 91, 60, 100, 57, 100 and 40 p.c. respectively. On restoration to saline the relaxation was 50, 66, 50, 44, 75 and 60 p.c. respectively. Higher concentrations abolish relaxation if the muscle is not rendered inexcitable.

Effect of monovalent anions

Bromide and nitrate increase relaxation, but if they cause tonic contraction, they decrease it. Thus in 6 muscles, the relaxation in saline was 55, 76, 80, 70, 68 and 64 p.c. respectively. In bromide saline it was 50, 47, 80, 50, 50 and 66 p.c. respectively. On restoration to saline, it was 58, 43, 50, 30, 33 and 23 p.c. respectively, withdrawal of bromide further diminishing the relaxation in 5 muscles. In another 6 muscles, the relaxation in saline was 45, 83, 73, 77, 89 and 81 p.c. respectively. In nitrate saline it was 66, 83, 83, 83, 71 and 60 p.c. respectively. On restoration to saline it was 25, 100, 80, 50, 80 and 100 p.c. respectively. Iodide has similar action. Thus in 6 muscles, the relaxation in saline was 25, 50, 50, 80, 39 and 36 p.c. respectively. In saline, in which 50 p.c. of the chloride was replaced by iodide, the relaxation was 33, 50, 50, 0, 64 and 62 p.c. respectively. In saline in which all the chloride was replaced with iodide, the relaxation was 50, 50, 0, 0, 40 and 50 p.c. respectively. In saline again, the relaxation was 80, 62, 20, 0, 50, 80 p.c. respectively. Thiocyanate mostly increases relaxation, as tone is decreased, but an opposite effect is produced if tone is increased. Thus in 6 muscles, the relaxation in saline was 75, 44, 75, 33, 88 and 61 p.c. respectively. In saline, in which 50 p.c. of the chloride was replaced with thiocyanate, the relaxation was 72, 77, 84, 54, 83 and 44 p.c. respectively. In saline in which all the chloride was replaced with thiocyanate, the relaxa-

tion was 100, 86, 100, 86, 80 and 51 p.c. respectively. In saline again the relaxation was 50, 82, 66, 70, 71 and 100 p.c. respectively.

Effect of drugs

Effect of acetylcholine.—Small concentrations may increase or decrease relaxation, but large concentrations which cause tonic contraction, decrease relaxation. Thus in 6 muscles, the relaxation in saline was 74, 78, 64, 75, 75 and 66 p.c. respectively. In acetylcholine (1 in 10^7), the relaxation was 71, 72, 57, 54, 87 and 68 p.c. respectively. In acetylchloine (1 in 10^6), the relaxation was 86, 42, 68, 58, 80 and 60 p.c. respectively. In acetylcholine (1 in 10^5), the relaxation was 40, 50, 50, 0, 66 and 0 p.c. respectively. In saline again it was 0, 0, 25, 16, 66 and 50 p.c. respectively. Acetylcholine diminishes the relaxation of contraction produced by potassium. Thus in 6 muscles stimulated with potassium saline, the relaxation was 44, 50, 100, 100, 100 and 55 p.c. respectively. After treatment with acetylcholine (1 in 10^5), the relaxation was 27, 0, 0, 0, 50 and 33 p.c. respectively. These effects resemble those on contraction.

Effect of adrenaline.—Small concentrations may increase relaxation; large concentrations decrease it. Thus in 6 muscles, the relaxation in saline was 65, 66, 73, 72, 72 and 81 p.c. respectively. In adrenaline (1 in 10^6), it was 69, 77, 85, 100, 40 and 33 p.c. respectively. In adrenaline (1 in 10^5), it was 0, 50, 0, 0, 0 and 0 p.c. respectively. In saline again it was 66, 62, 50, 66, 100 and 50 p.c. respectively. These effects resemble those on contraction.

Effect of eserine.—Small concentrations increase and large concentrations decrease relaxation. Thus in 6 muscles, the relaxation in saline was 86, 71, 60, 72, 72 and 71 p.c. respectively. In eserine (1 in 10^7) it was 88, 77, 63, 78, 76 and 43 p.c. respectively. In eserine (1 in 10^6) it was 50, 46, 25, 36, 28 and 33 p.c. respectively. In eserine (1 in 10^5), it was 37, 55, 16, 50, 20 and 0 p.c. respectively. In saline again, it was 0, 100, 50, 0, 0 and 50 p.c. respectively. Large concentrations may permanently impair relaxation. These effects resemble those on contraction.

Effect of pilocarpine.—Pilocarpine causes tonic contraction and so decrease relaxation. Thus in 6 muscles, the relaxation in saline was 72, 39, 26, 41, 48 and 29 p.c. respectively. In pilocarpine (1 in 10^6), the relaxation was 8, 14, 25, 0, 50 and 50 p.c. respectively. In pilocarpine (1 in 10^5) the relaxation was 0, 0, 0, 0, 0 and 33 p.c. respectively. In saline again, it was 75, 0, 40, 0, 0 and 0 p.c. respectively. This effect resembles that on contraction.

Effect of nicotine.—Nicotine may increase relaxation in small concentrations, and decrease it in larger ones. Thus in 6 muscles, the relaxation in saline was 42, 60, 50, 38, 38 and 52 p.c. respectively. In nicotine (1 in 10^6) it was 61, 0, 50, 0, 75 and 65 p.c. respectively. In nicotine (1 in 10^5), it was 0, 43, 28, 50, 33 and 40 p.c. respectively. In saline again, it was 53, 0, 54, 44, 0 and 56 p.c. respectively. These effects resemble those on contraction.

Effect of methyl alcohol.—This has a curious effect. Small concentrations may decrease, but larger concentrations may increase relaxation. Thus in 6 muscles, the relaxation in saline was 84, 75, 40, 61, 48 and 31 p.c. respectively. In methyl alcohol (1 in 1,000), it was 80, 30, 57, 80, 30 and 14 p.c. respectively. In methyl alcohol (1 in 100), it was 100, 40, 58, 100, 66 and 57 p.c. respectively. In saline again, it was 100, 100, 46, 75, 83 and 80 p.c. respectively.

Ethyl alcohol has similar effects. Thus in 6 muscles, the relaxation in saline was 64, 50, 63, 89, 78 and 60 p.c. respectively. In ethyl alcohol (1 in 1,000), it was 85, 70, 43, 100, 54 and 40 p.c. respectively. In ethyl alcohol (1 in 100), it was 0, 57, 66, 75, 100 and 0 p.c. respectively. In saline again it was 66, 0, 0, 18, 0 and 0 p.c. respectively. Effects on contraction produced by alternating current are the opposite; these effects, however, resemble those on contraction produced by potassium (Singh and Singh, 1946 *b*).

Effect of osmotic pressure

Decrease in osmotic pressure at first increases and then abolishes relaxation: Thus in 6 muscles, the relaxation in saline was 50, 88, 54, 25 and 53 p.c. respectively. If the osmotic pressure was decreased to 80 p.c. of normal, the relaxation was 78, 54, 85, 42, 36 and 50 p.c. respectively. In osmotic pressure, 60 p.c. of normal, relaxation was 33, 33, 50, 40, 0 and 60 p.c. respectively. In osmotic pressure 40 p.c. of normal the relaxation was 0, 0, 0, 0, 0 and 0 p.c. respectively. In saline again, the relaxation was 30, 100, 75, 83, 87 and 40 p.c. respectively. Increase in osmotic pressure has similar effects. Thus in 6 muscles, the relaxation in saline was 71, 69, 52, 54, 78 and 50 p.c. respectively. In saline the osmotic pressure of which was increased to 120 p.c. of normal by adding sodium chloride, the relaxation was 74, 51, 82, 40, 50 and 42 p.c. respectively. In osmotic pressure, 140 p.c. of normal, the relaxation was 55, 35, 40, 43, 66 and 62 p.c. respectively. Further increase of osmotic pressure rendered the muscle inexcitable.

An interesting point is that if the muscle is immersed in a hypertonic solution, restoration of normal osmotic pressure diminishes or abolishes excitability. Thus the muscle adapts to the new osmotic medium. Simi-

larly if frog's muscle is immersed in the electrolytic free-medium, restoration to saline is toxic.

Tonus

Substances that damage the muscle cause tonic contraction (Singh and Singh, 1946 *b*). If the muscle is in a state of active inhibition, then anything that depresses its vitality will cause tonic contraction which will not be abolished by asphyxia. This is well shown in Figs. 4, 5; poisons such as

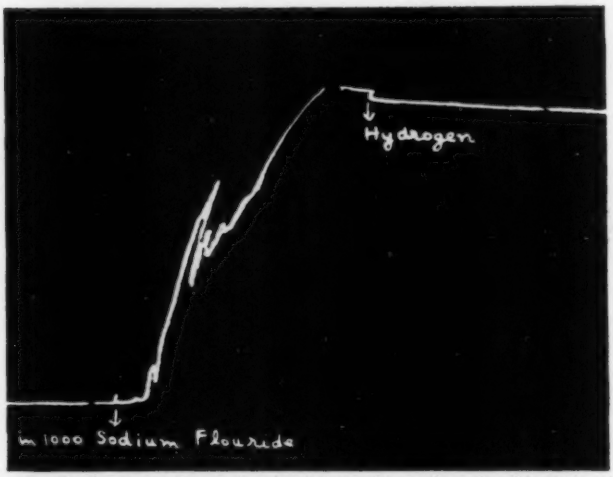


Fig. 4. Dog's stomach muscle stimulated with sodium flouride (1 in 1000).

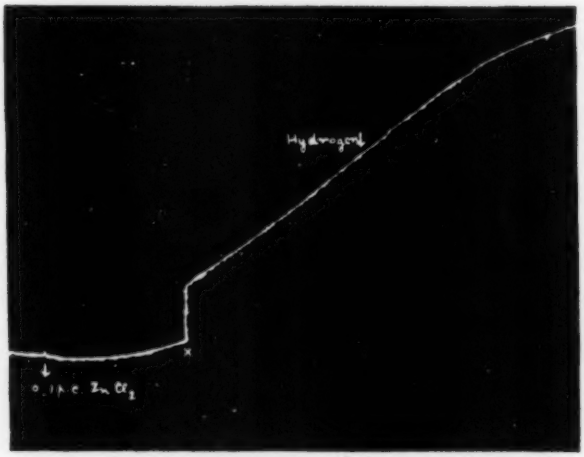


Fig. 5. Dog's stomach muscle stimulated with 1% $ZnCl_2$.

zinc, mercury, fluoride and anæsthetics such as chloroform, urethane cause contraction. A well-known depressant such as magnesium causes contraction. Cold also causes contraction due to similar cause.

These depressants and poisons also cause an initial contraction through the excitatory process. This contraction develops immediately on adding the substance, and is abolished by asphyxia (Fig. 6); the other contraction develops slowly after a few minutes. In Fig. 7 it will be seen that chloro-

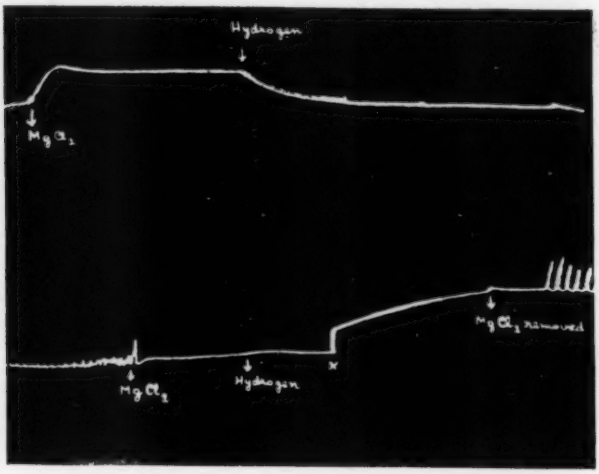


FIG. 6. Dog's stomach muscle stimulated with magnesium saline. Upper curve shows contraction susceptible to asphyxia. Lower curve shows contraction resistant to asphyxia. After removal of magnesium, muscle stimulated with alternating current.

form causes an initial contraction. Asphyxia and cyanide also produce similar effects (Singh, 1949).

The explanation of the action of these substances on the excitatory process appears to be as follows. Normally there is a balanced action by excitatory and inhibitory ions inside and outside the muscle fibres. A substance which depresses vitality may depress the action of inhibitory ions, thus causing excitation. There are 16 different ways in which excitation or inhibition may be produced in unstriated muscle (Singh, 1942; 1944 *c*).

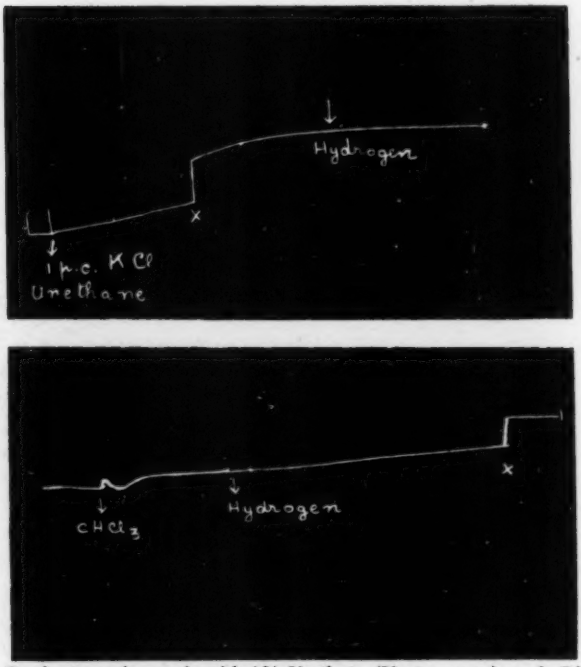
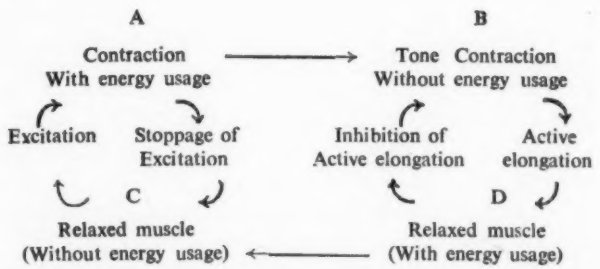


FIG. 7. Dog's stomach muscle with 1% Urethane (Upper curve) and Ringer, saturated with chloroform (Lower curve).

A scheme for unstriated muscle

The reactions of unstriated muscle may be represented by the following cheme.



The diagram suggests that there are two kinds of relaxed state, one using energy and the other not. This is shown by the fact that when a muscle dies, it does not always contract; asphyxia or iodoacetic acid does not always produce contraction.

Let us now see how far this scheme is supported by experimental evidence. In Fig. 8, the muscle is stimulated with 0·06 *M* KCl. It is then

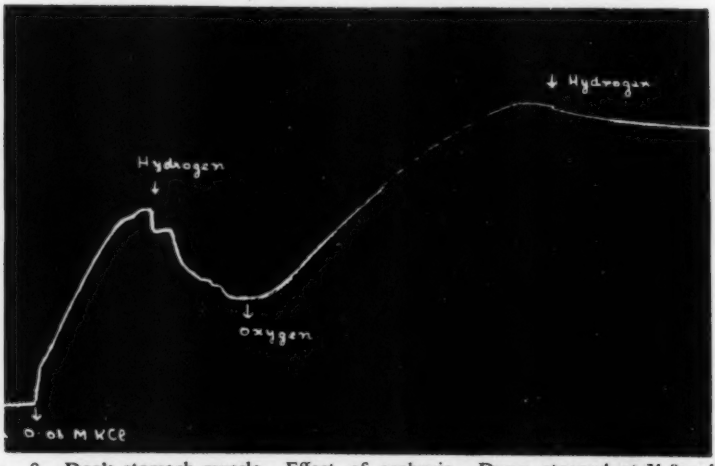


FIG. 8. Dog's stomach muscle. Effect of asphyxia. Drum stopped at X for development of tone.

asphyxiated. This shows that to start with, the muscle was in state C; it then passed to state A. On reoxygenation the tension rises again, above the previous value, as asphyxia makes the muscle more sensitive to oxygen (Singh, 1949). The muscle does not relax again on asphyxiation, therefore it has passed to the stage B. This experiment therefore shows the path CAB.

In Fig. 9 the muscle is stimulated with potassim. It first elongates; this has been shown to be active. Therefore to start with the muscle was

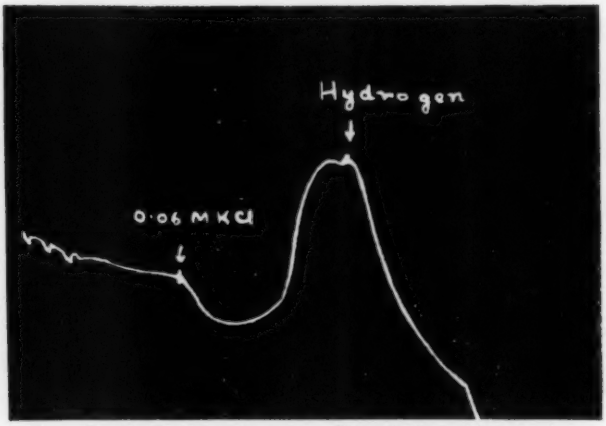


FIG. 9. Dog's stomach muscle. Stimulation with potassium.

in state B. It begins to contract; this contraction is abolished by asphyxia; therefore the muscle had passed at first to the stage D and then A. It must have therefore passed through stage C. This experiment therefore shows the path BDCA.

In Fig. 1 the muscle when stimulated with potassium, at first elongates, then contracts and further accommodates, which process has been shown

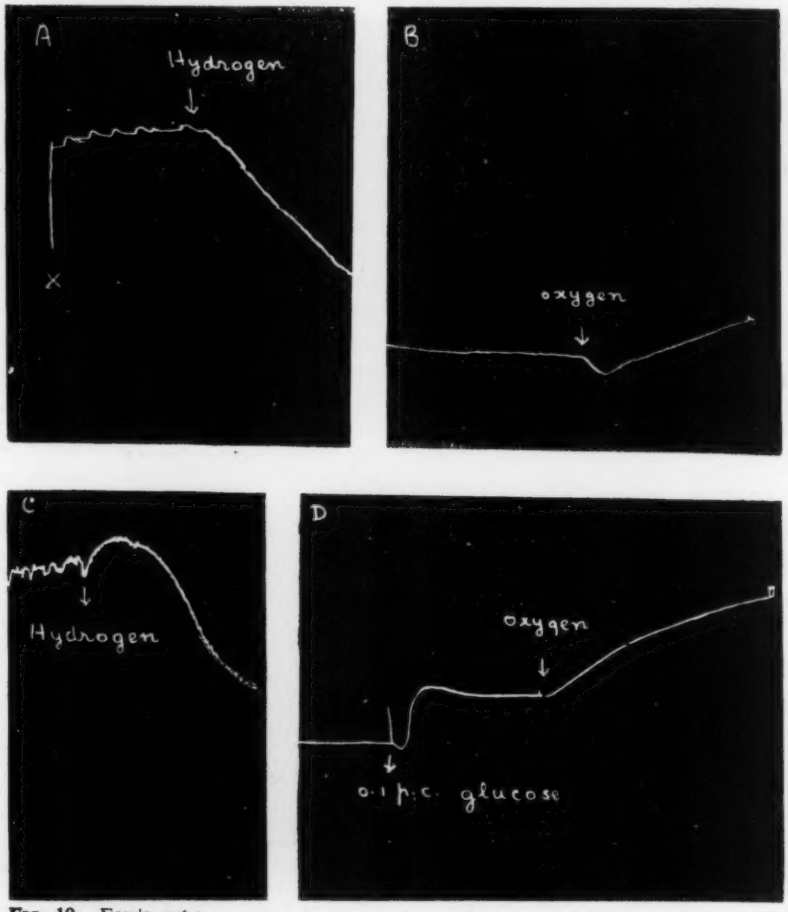


Fig. 10. Fowls gut :

A. Muscle asphyxiated.
B. When it had completely relaxed oxygen is introduced.
C. Muscle asphyxiated.
D. When it had completely relaxed, 0·1 p.c. glucose added.

to be active. Therefore the muscle to start with was in stage B, it then passes to the stage D, then to stage C, then to A. As it is actively elongating, from the stage A, it must have passed to B, and then is passing to stage D. This experiment therefore shows the path BDCABD. The path AC is shown when the muscle relaxes, and the path DB when the muscle contracts under the influence of iodoacetic acid.

In Fig. 10 A, B, the muscle was asphyxiated and then oxygenated; oxygen at first produces inhibition and then increases tone. This figure then shows the path CABDCA, the state B occurring with the muscle relaxed. In Fig. 10 C, D the same effect is produced by glucose as oxygen in Fig. 10 B. Thus in the presence of glucose, oxygen loses its inhibitory effect (Singh and Singh, 1948 *c*; Singh, 1949); these effects are explained by the above scheme; it therefore appears that the path BA does not occur. B is converted to A only by the route BDCA.

The fact that the state B may occur in a relaxed muscle is of importance in that it may represent normal tone of the muscle which does not use oxygen. Unstriated muscle is never relaxed in the sense that striated muscle is; its relaxation is merely a change in the degree of contraction. In some muscles, glucose alone may not be able to change the state B (Fig. 11). Nothing is known regarding the path CD.

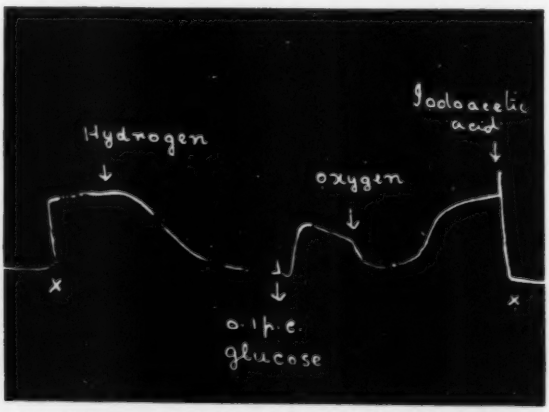


FIG. 11. Guinea pig's uterus. Effect of asphyxia, glucose, oxygen and iodoacetic acid (1 in 20,000), Drum stopped at X.

DISCUSSION

In unstriated muscle there appear to be two cycles. In one cycle the energy for contraction is derived from chemical stores. In the other cycle

the energy for contraction is derived from energy previously stored in the structure. These two cycles account for the two kinds of tones in unstriated muscle, one that uses energy and the other that does not do so.

These experiments show that one of the functions of the glycogen lactic acid system is to keep the muscle relaxed. There appear to be two different kinds of contractile proteins, one of which is relaxed and the other contracted, or in the same protein there are chemical groups which are acted upon differently by production of lactic acid. It would appear that the normal state of the muscle whether in relaxation or contraction is a dynamic equilibrium between two opposing forces. This might account for the presence of a dual nerve supply to unstriated muscle.

Thus relaxation would occur if excitation by excitatory nerves is stopped, or if the inhibitory nerves are brought into action. This also suggests that the tone of the muscle is not dependent upon continued action of the excitatory nerves. If this were so, then the presence of inhibitory nerves would be superfluous. In the third paper, experiments will be described to dissociate the two contractile elements of unstriated muscle.

Summary

1. Dog's stomach muscle and the human appendix relax actively after being stimulated.

2. Dog's stomach muscle relaxes actively during inhibition and accommodation.

3. Relaxation is of two kinds, active and passive.

4. Active relaxation is diminished by asphyxia, cyanide and partially restored by glucose.

5. Active relaxation is diminished by iodoacetic acid.

6. Active relaxation is diminished by substances that produce tonic contraction, such as potassium, ammonium, lithium, sodium, barium, hydrogen, ions, calcium, strontium, magnesium, bromide, nitrate, iodide, thiocyanate, acetylcholine, pilocarpine, nicotine, eserine, adrenaline.

7. Methyl and ethyl alcohols diminish active elongation in small and increase in large concentrations.

8. Active elongation is diminished by hypo- and hypertonic solutions.

9. The optimum pH for active elongation is 8.

10. The optimum temperature for active elongation is 30° C.

11. Substances that depress the vitality of the muscle cause contraction by antagonising active elongation.

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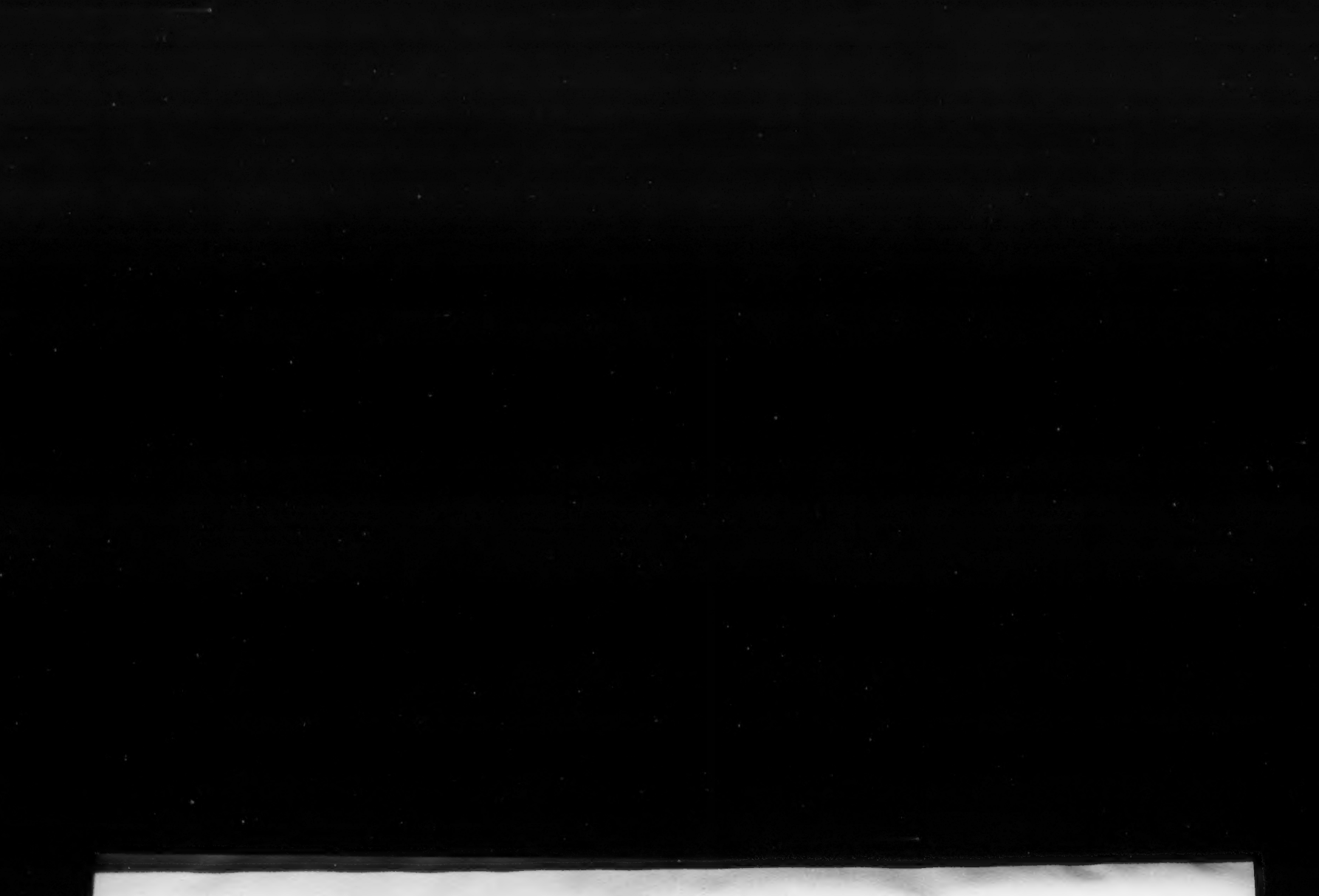
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